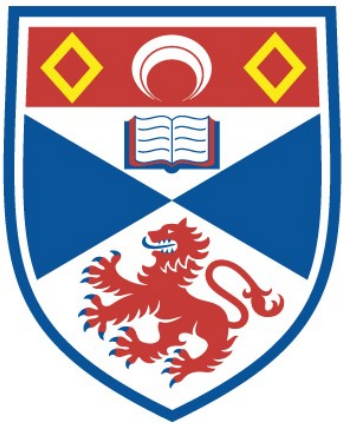
THE STRUCTURE AND FUNCTION OF CERTAIN NEUROMUSCULAR SYSTEMS IN ECHINODERMS

James Leslie Stiles Cobb

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1967

The Structure and Function of Certain Neuromuscular Systems in Echinoderms.

By

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Thesis submitted for the Degree of Doctor of Philosophy.

DECLARATION

I hereby declare that the work recorded in this thesis has been carried out by myself, and that it is of my own composition. I further declare that it has not been submitted in any previous application for a higher degree.

I certify that James Cobb has fulfilled the conditions laid down in the regulations for a degree of Doctor of Philosophy, under Ordinance No. 16 of the University Court of the University of St. Andrews and that he has accordingly qualified to submit this thesis for the Degree of Doctor of Philosophy.

Vitae.

I was educated at Christ's College, Brecon and attended University at St. Andrews where I graduated in Honours Zoology in 1964. The work described in this thesis was carried out between October 1964 and July 1966.

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Footnote:-

All illustrations in this thesis are photocopies of the originals.

Summary.

1. Physiological experiments were devised to study the electrical responses of echinoderm muscle during contraction. The smooth muscle forming the lantern retractor muscles of Echinus conducts spike potentials at a speed of 4 cms. a sec.

2. Three muscle systems examined with the electron microscope reveal a layout that varies in complexity. The muscle cells give rise to processes that are synapsed upon by nerves.

3. The area containing the ribbon axons described by Smith (1950) was examined and the ribbon axons shown to be selectively stained muscle cells.

4. In the three muscle systems examined a controlling ganglion was found to be associated with each separate organ.

5. The ganglia contained large numbers of structures that are described as synapses. These synapses are of a simple type and do not possess the specialisations that are present in the synapses of some other invertebrate phyla.

6. The sensory cells of the epithelium are described and an examination has indicated that the cells of the general epithelial are all sensory and contribute axons to the nerve plexus. Some of these epithelial cells are modified into probable chemoreceptors, light receptors and are found in statocysts.

7. A hypothesis to account for the co-ordination of the behaviour of echinoderms is propounded.

ACKNOWLEDGEMENTS

I should like to thank Dr. M.S. Laverack for the great deal of help and encouragement he has given to me during the years that I worked in the Gatty Marine Laboratory on this work. In particular I am grateful for his pointing out that Philosophy is not spelt with an 'F'.

I should also like to record my gratitude to John Mackie for patient help with electron microscopy and John Stevenson for many hours spent sorting out photographic problems. The help of Dr. G.A. Horridge and other members of the Gatty community with a diversity of problems is much appreciated.

I am grateful to Her Majesties Government for financing me, and to the echinoderms for being enigmatic.

"He laments, Sir, his student goes a-birding".
with apologies to W. Shakespeare.

INTRODUCTION

Some species of echinoderms were well known to both the Romans and the Greeks and figure in a number of works written almost two millenia ago. They have not always been described as a separate phylum but have featured in many mediaeval bestiaries. Since the middle of the last century they have been regarded as a major invertebrate phylum.

The classical histological studies on which our present knowledge stands were carried out at the end of the 19th century, the most important being those of Preuho (1887) and Hamann (1887). Von Uexkull working at about the same time described much of the behaviour of echinoderms and the results of simple physiological experiments carried out on them. Although the echinoderms have attracted attention from many other workers since, the amount of work has been meagre when the size and importance of the phylum is considered. Descriptions of both physiological and histological aspects of echinoderm biology have been published.

The only major histological work which amplifies the findings of the late 19th century is that of Smith, 1937, 1947 and 1950. This work was carried out using methylene blue staining, dissection and normal histological techniques and describes much of the innervation of the muscle systems of asteroids. It is most noteworthy that the echinoderms have

received so little attention from anatomists. The main reason for this becomes obvious to those who attempt to study the group; it is that they offer little in the way of tangible results for long hours spent in examining them. This is chiefly due to spicules of calcium carbonate that invade most of the tissue and make histological sectioning most difficult. Further the tissues of echinoderms do not always react predictably to staining techniques that are well tried on other groups of animals.

The results of some of the most important work on the physiological properties of nerve/muscle systems were published in a series of papers by Boltt and Ewer (1963) and Pople and Ewer, (1954, 55 and 58). In these works more than any other an attempt has been made to correlate modern physiological concepts with echinoderm behaviour. Prosser and associates in a series of papers, (1951, 54 and 65) have examined the properties of echinoderm muscle in experiments comparing the behaviour of various invertebrate phyla. These results indicated that certain muscles in echinoderms could function as a block without the interaction of nerves. No description was given of the system in detail however.

Millett and various co-workers, (see 1960, a and b, and 1963) have described the response of echinoids, in

particular Diadema, to light, examining the shadow response of these animals and the role of the peripheral nervous system and that of the nerve cords in co-ordinating the behaviour. However since the anatomical details of the nervous system are not known and the observed response extremely complex it has not been possible to draw firm conclusions about the co-ordinating system. However many interesting facts about the capabilities of the nervous system have been demonstrated.

Apart from his histological work on echinoderms Smith (1950) has also published findings on the behavioural responses of this phylum. This work chiefly describes the co-ordinated behaviour of starfish locomotion and the role of the nervous system. Kerkut (1953) amplified this work and taken together it provides about the only good example of a description of the co-ordination of a major behavioural response of the whole of an echinoderm and the effects of experiments designed to interfere with the normal behaviour. The work acts as a clear pointer to some features that are emerging as typical of echinoderm co-ordination.

Sandeman (1965) and Takahashi (1965) both have published details of the electrical activity of the nervous system of echinoids and these remain the only published observations. The former described compound electrical potentials and the latter unitary activity. They are discussed at length later. Cobb and Laverack, (1966) have published one paper, the results of which are described in

full later. Significant unpublished findings on the electrical activity within the muscle, Cobb, are also described in full.

There has also been a certain amount of work on the neuropharmacology of echinoderms, (see Welsh, 1966) and the function of other organs, but as this is outside the present consideration of neuromuscular systems and co-ordination they will not be referred to within the context of this thesis.

In the last decade or so the electron microscope has enabled the morphological detail of nervous systems to be examined at a completely new level of resolution. There have been relatively few electron microscope studies of echinoderms, however these studies have shed new light on certain aspects of the nervous anatomy and shown the interpretation of earlier histological studies to be misleading in some cases.

Bargmann and Behrens (1963) worked on the ampullae of an asteroid in an endeavour to confirm features of the nervous system described by Smith (1950). They failed however to confirm the description by Smith and also failed to demonstrate any nervous elements associated with the muscles of this organ.

Eakin and Westfall, (1964) and Eakin (1963) described the fine structure of the ocelli of asteroids, as did von Harnack, (1963). Unger, (1962) described features of the nervous system that he considered were associated with neuro-secretion. The work of Eakin and Westfall (1964) compares interestingly with the study of another sensory area made during

this study Cobb (1968) and with observations of Laverack (1968) on the statocyst of a holothurian. Kawaguti (1964, 1965) has published an extensive series of papers on echinoderm fine structure covering a wide range of topics. These studies are very incomplete in detail, dealing only superficially with the problems. Further many of the statements in the text, though interesting, and possibly correct, are not supported by pictorial evidence, and even where they are, the evidence is very poorly presented.

The work reported in this thesis consists of the physiological work mentioned earlier and a number of discrete electron microscope studies. Three complete nerve/muscle systems are described. The innervation of the lantern retractor muscles and the attendant ganglion is described, this preparation was studied in the urchin Echinus esculentus, as was the innervation of the smooth and striped muscles of the pedicellariae. In the pedicellariae preparation, the sensory system of the jaws and the relationship of the sensory axons from these areas to the controlling ganglia of the pedicellariae were described. The third nerve/muscle preparation described was that of the muscles of the ampullae of Astropecten irregularis. This nerve/muscle preparation was first described by Smith (1950) from methylene blue staining of living material and the electron microscope studies

have shown this work to be in need of re-interpretation. A study of the radial cord of *Astropecten* was made in detail using the electron microscope and the results compared with a briefer study of the radial cord of *Echinus*.

Physiology

Methods and Materials

Sea urchins (Echinus esculentus) were obtained from two sources; from the creels of local lobster fishermen, and by skin-diving members of the Forth Valley Subaqua Club. The animals were maintained in large aerated tanks with circulating sea water. They remained suitable for use only if kept under optimum conditions of temperature and aeration. Interruptions of sea water flow or aeration had drastic effects upon their survival in aquaria; the temperature should be held in the range 2 to 10°C according to the season.

The anatomical description (Cobb 54) differs somewhat from that of previous workers. The details were elucidated by simple dissection of the lantern together with concurrent staining by methylene blue (rongalit reduced).

Early experiments on the physiology of the lantern musculature were carried out using conventional methods for kymograph recording. The muscles were left in situ, a small glass hook being placed underneath the horizontal retractor muscle, a thread leading to the writing lever being under just sufficient tension to enable muscle contractions to be recorded on the kymograph. Cutting the muscle for attachment of the thread to one end proved too traumatic and preparations did not survive. Experiments have shown that the muscles are extremely sensitive to stretch.

The stimulating electrodes used were usually thin platinum wires but direct stimulation of the muscle required application of the electrodes to the muscle surface, and this was often followed by local retraction of the muscle away from the electrodes. In later experiments therefore sharpened stainless steel pins were inserted into the muscle. Stimulation of the radial nerve cord was accomplished by raising the cord on to platinum wire hooks. In cases in which the hyponeural tissue was stimulated, two fine silver wires carried on a fine glass thread, and with a very small gap between them, were applied to the area, these were insulated with varnish.

In all experiments the cut test (oral half) of the urchin acted as a chamber for the perfusion of the muscles with sea water. Under such conditions preparations remained viable for many hours.

Later physiological experiments were carried out using intracellular and extra-cellular micro-electrode recording techniques.

Extra-cellular techniques

In all the extra-cellular recording experiments a standard differential amplifier was used connected to a Tetronix 561A oscilloscope. Photographical records of electrical activity were recorded directly using a Gossor 1431 camera attached to the oscilloscope. In experiments requiring a stimulus, single stimuli of variable pulse length

and amplitude were delivered using a Tetronix Type 161 pulse generator triggered using a 9 volt battery and a flash contact switch. A number of different techniques were experimented with on various preparations. Only one technique on a particular preparation provided satisfactory results. Efforts were made to record from both nerve and muscles using different variations of silver and platinum wire electrodes. It was however impossible to detect any electrical activity using these types of electrodes.

Further experiments were initiated using indium-filled glass electrodes and also electrolytically sharpened tungsten needles insulated with varnish. Again neither in muscle preparations nor in nerve preparations could electrical activity be recorded. In two preparations in particular some considerable effort was made to achieve success in order to confirm the results of Takahashi, (1964). These were the radial cord of Astropecten irregularis and the hyponeural ganglion of Echinus esculentus. No satisfactory results were however achieved.

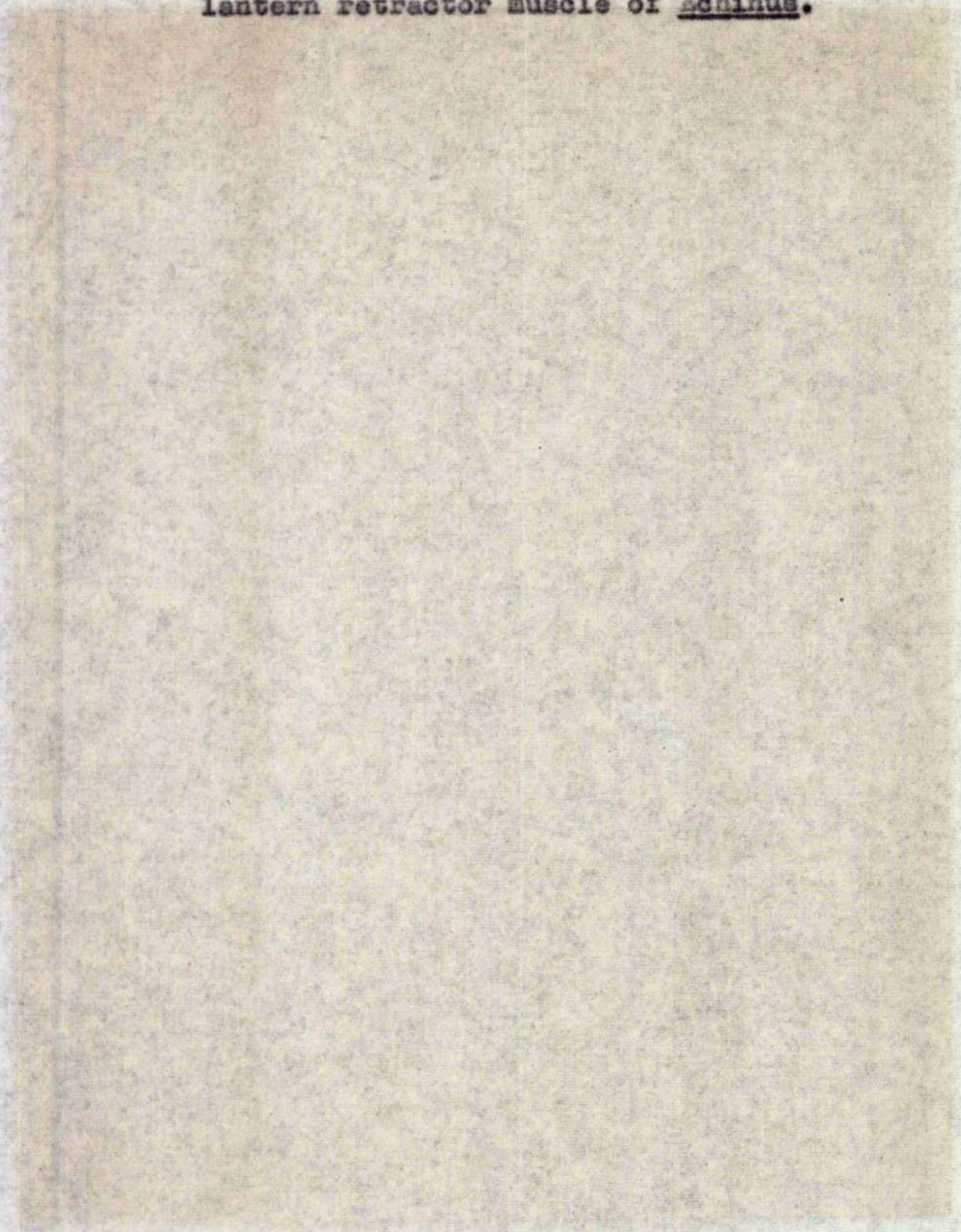
Finally both glass and plastic suction electrodes were experimented with. The glass electrodes were later abandoned in favour of the plastic suction electrodes. When the plastic suction electrodes were used to record from nerve once again no satisfactory results were obtained. When placed on the retractor muscles of Echinus however electrical activity was

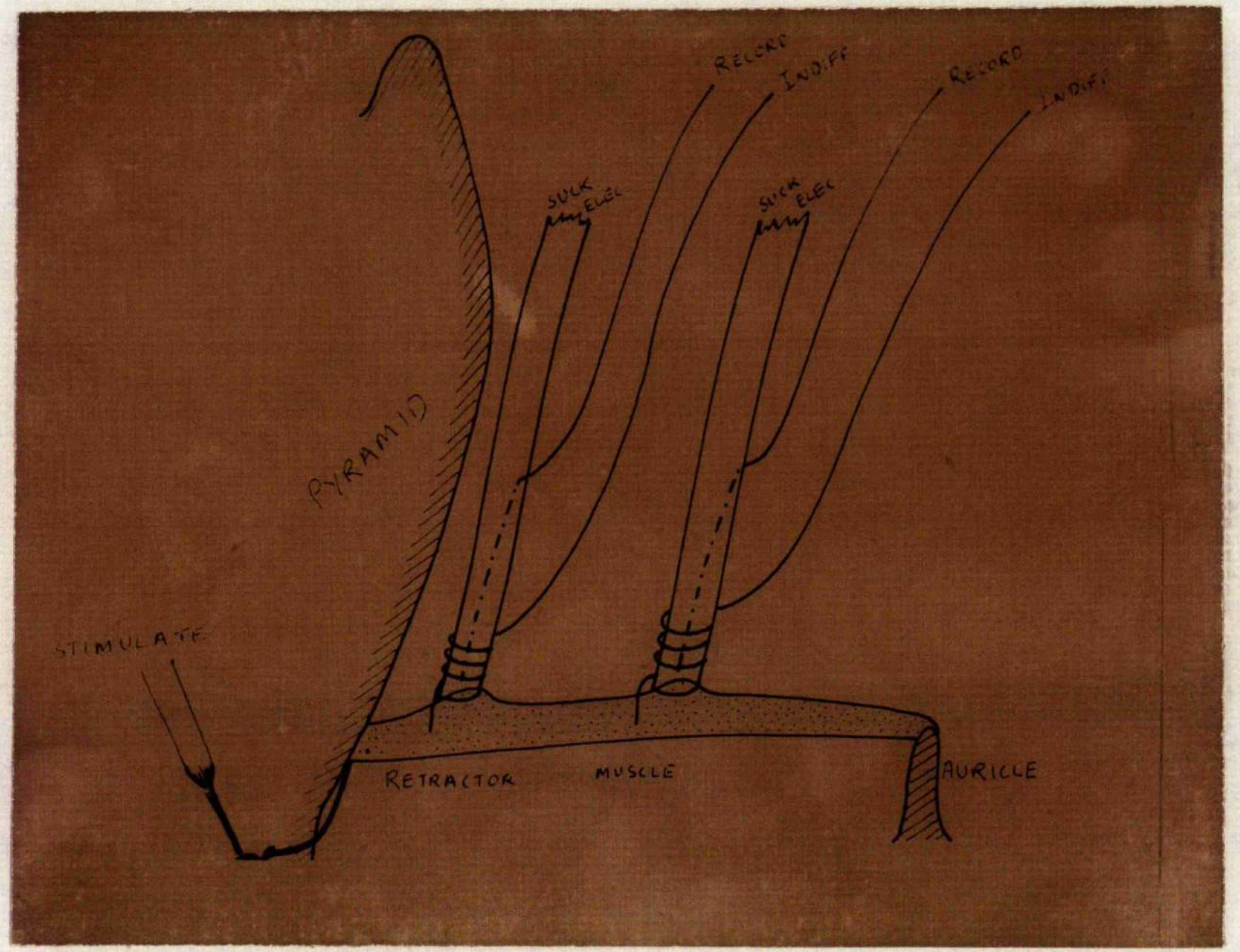
observed. The apparatus used to record from this last preparation is illustrated in Figure 1. The stimulating electrodes were placed in the hyponeural ganglion described in Cobb (1964). The hyponeural ganglion was dissected out using a high speed air-bearing dental drill. The use of this type of drill enabled the pyramids to be cut just above the hyponeural ganglion without causing any shattering of the calcareous parts or tearing the tissue overlying them. The muscle itself was left intact after the coelomic membrane had been dissected away. In large urchins the retractor muscle is long enough to enable the electrode to be moved into at least a dozen positions or, as in later experiments, to enable two suction electrodes to be placed on the muscle separated by up to five millimetres. The placing of two electrodes on the muscle allowed the rate of conduction of the electrical activity up the length of the muscle to be measured.

Intra-cellular electrodes.

Early attempts to record intra-cellular activity met with little success. 3 molar KCl was used to fill glass micro-electrodes. The electrodes used varied from 20 megohms resistance to 50 megohms. A standard Bak amplifier was used connected to a Tetronix S61A oscilloscope.

Fig. 1. Diagram illustrating the apparatus used to record extra-cellular potentials from the lantern retractor muscle of *Echinus*.




RECORD
INDIFF
RECORD
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SUCK ELEC
SUCK ELEC
PYRAMID
STIMULATE
RETRACTOR MUSCLE
AURICLE

Results

Cobb (1964) reported a nerve muscle preparation using the lantern retractor muscle of Echinus. The early experiments described have been repeated using more closely controlled stimulating parameters but still recording the movements of the muscle using a kymograph. The responses of the whole lantern to stimulation and the spontaneous rhythms are described in Cobb and Laverack 1966 as are the responses to direct stimulation of the muscle with electrical pulses.

Stimulation of the retractor muscle via the hyponeural tissue, (i) mechanical responses of the muscle.

Control of muscles is via a complex innervation as outlined above. When some of the lantern pyramids are dissected away it is possible to place electrodes on the circumoral ring for the purpose of electrical stimulation. In yet other experiments the pharynx was severed at the level of the circumoral ring to allow stimulation of the hyponeural tissue.

These plaques of nervous tissue should be considered as complex ganglia (Cobb & Laverack 1966a).

Electrical stimulation of the circumoral ring, carried out with silver electrodes placed on or in the ring, is without effect on the retractor muscles; no contraction is observed.

Hyponeural stimulation is followed by marked contraction of the retractor muscle. The responses vary

according to the site of stimulation within the ganglion, and with the variation in frequency, pulse length and intensity of the applied shocks.

(a) Frequency

When square pulses above threshold are applied at a constant intensity and duration the frequency becomes the governing factor. At frequencies from 1 to 10 c/s there is a gradual increase in size of contraction; above this frequency there is a decrease from the maximum (figure 2). The reduction from the maximum becomes more marked with further increases in frequency (figure 2).

When very low frequencies are used the muscle shows a separate contraction per stimulus at 1 every 2 s, gradually summating as the frequency rises to fuse into a smooth twitch at 1 shock/sec and upwards. There is no facilitation, only mechanical summation.

(b) Intensity

Responses can be obtained from this preparation when using only 0.5 mA as stimulating current applied to the hyponeural tissue. Figure 2 shows the effect of altering current strength whilst other parameters are held constant.

(c) Pulse length

Variation in the pulse length from 0.5 to 5.0 ms duration, maintaining constant intensity and frequency, gives rise to graded responses (figure 3). The threshold to variation in pulse length is more sharply defined when compared to the

Fig. 2. Responses of retractor muscles to indirect electrical stimulation via the hyponeural ganglion.
(A) Increase in frequency from 1/s through 2, 4, 10, 20 to 50/s. Note reduction in amplitude of response above 10 c/s. (0.8mA, 5.0s).
(B) Effect of low-frequency stimulation. From left; response to physical stimulation of ganglion by approaching electrode, 20, 10, 5, 2, 1/s, 1 per 2s, 1 per 3s, 1 per 4s, 1 per 5 s and 1 per 10 s. (Note dual response in all records.)
(C) Variation of intensity. From left at marks, 0.2, 0.3, 0.5, 1.0, 2.0, 3.0 4.0 (5.0 ms at 10/s. for 2s.)
(D) Reduction of response at frequencies above 10/s (4, 10, 20, 50 and 100 c/s at 5.0 ms and 0.5 mA).

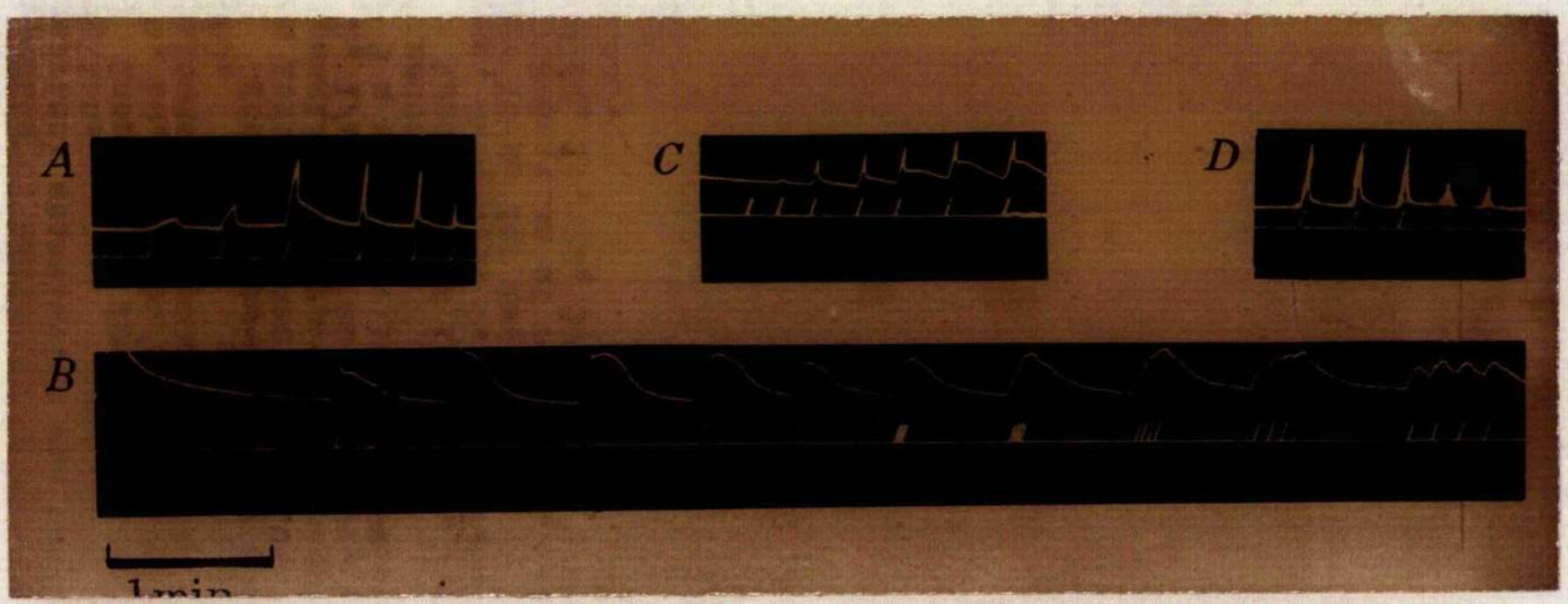
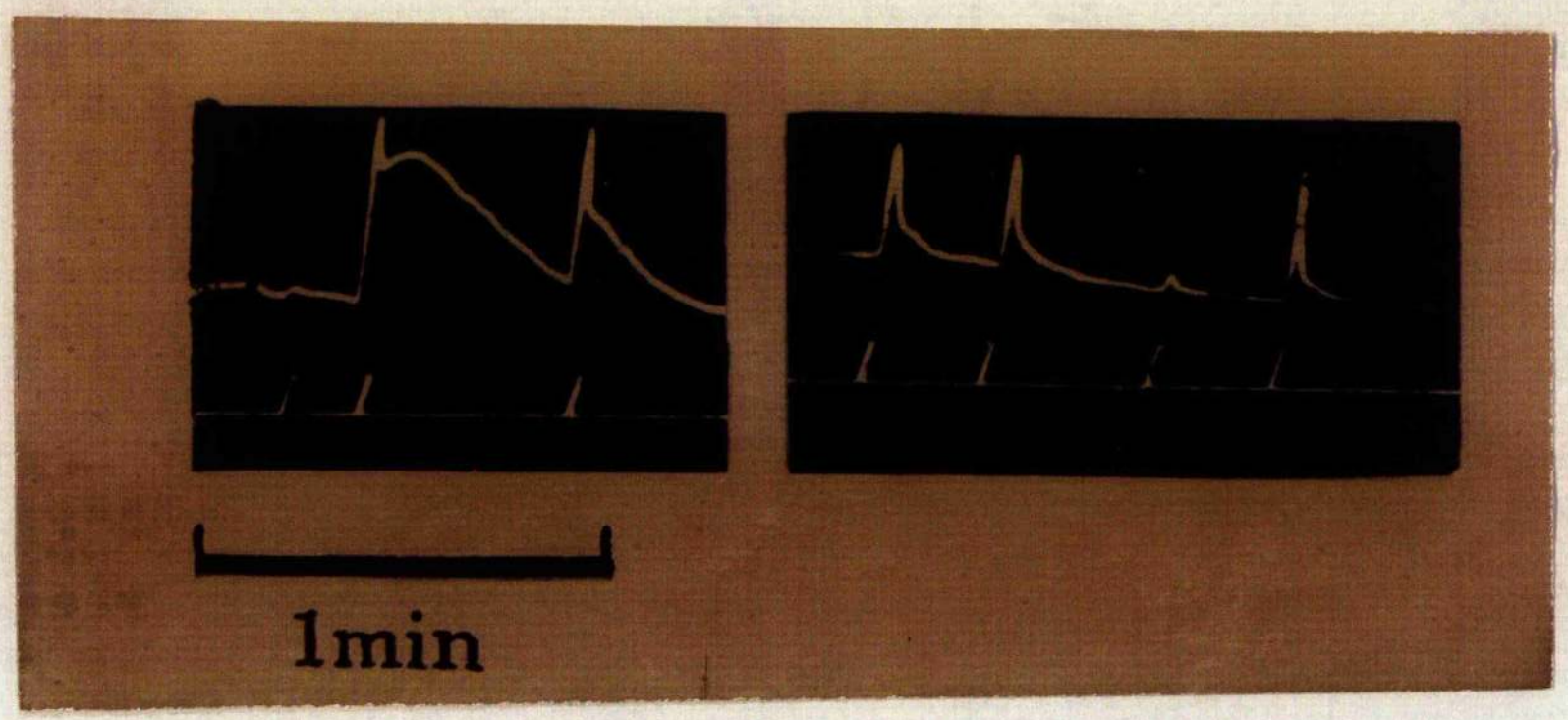

A
C
D
B
1 min

Fig. 3. (A) Pulse length also affects the retractor muscle response. From left 0.05, 0.1 and 0.2 ms (10 c/s at 6m.). (B) Pulse length from left, 0.2, 0.2, 0.1 and 0.2 ms. Note characteristic threshold effect.


1min

thresholds of responses to other parameters of stimulation.

The responses described above are examples of the fast contraction exhibited on stimulation of the hyponeural tissue. Under certain circumstances it was early noted that this was accompanied by a slower response. This delayed response occurred only when the electrodes were placed in a particular area of the hyponeural tissue.

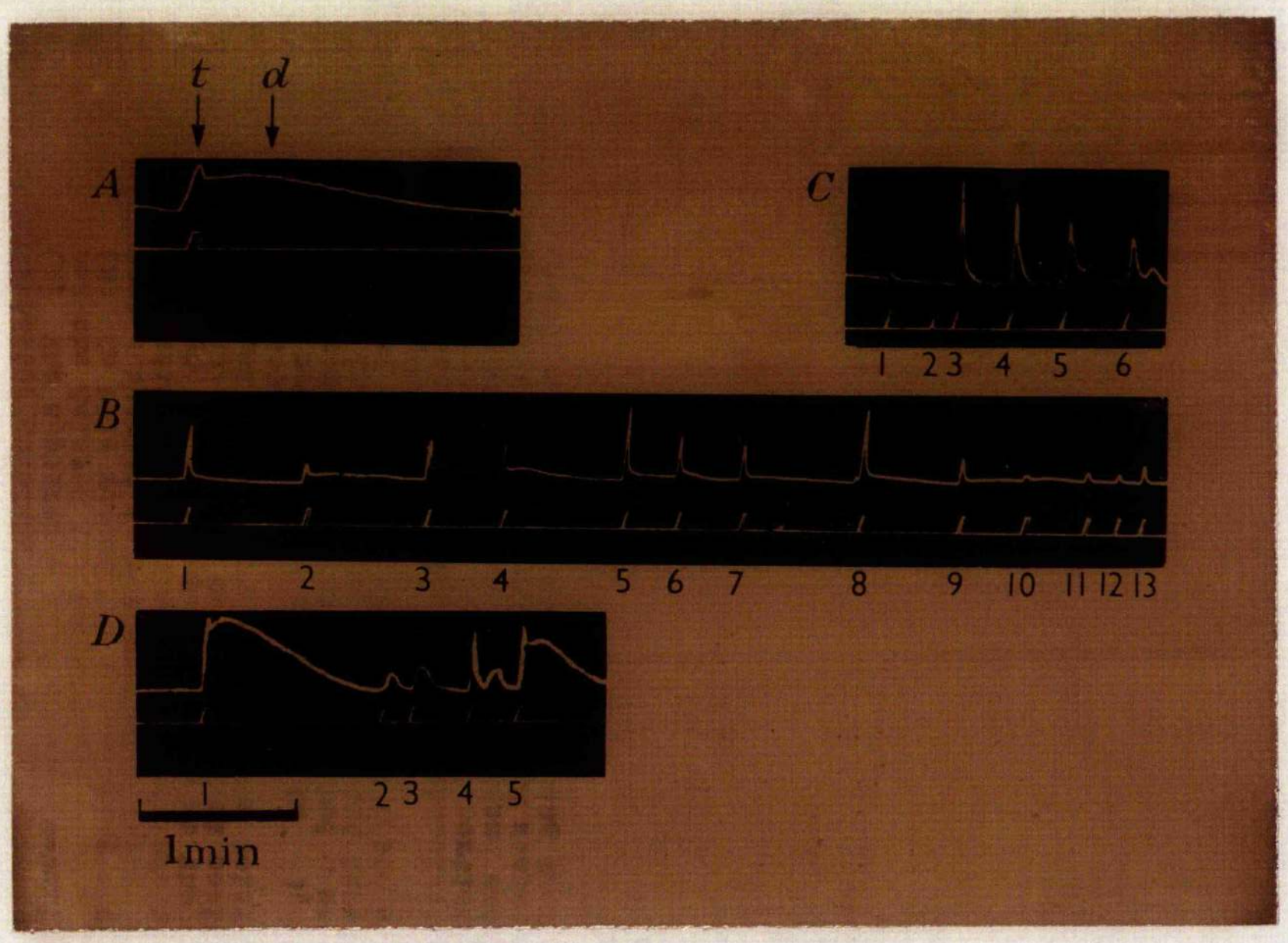
When current was passed, a rapid twitch muscle contraction resulted, followed within 500 ms by a slowly rising tension manifesting itself as a hump subsequent to the twitch (figure 4).

This response was always abolished when the hyponeural ganglion was destroyed. Stimulating one plaque of hyponeural tissue was followed by contraction only in the single muscle which it innervates.

Closely controlled stimulation of the hyponeural tissue shows that when efferent fibres are stimulated a fast response ensues. In the area where afferent fibres from the circumoral ring converge on the efferent pathways both fast and slow responses are elicited. If the electrode is placed distal to the proposed coordinating centre with respect to efferent pathways, then on some occasions only a delayed contraction occurs. Electrical responses of the muscle.

Many attempts were made (during this study) to record electrical potentials from the nerves and muscles of echinoderms. It eventually became clear however that by placing plastic

Fig. 4. Retractor muscles.
(A) The appearance of the fast (t=twitch) and the delayed (d) response.
(B) In this record the stimulating electrodes were subjected to reversal of polarity at each succeeding mark. The electrodes were advanced through the hyponeural ganglion as the experiment continued. It can be seen that at positions 2, 3 and 4 there are signs of the delayed response.
(C) A repeat of the experiment in 4B, but the electrodes in this case stimulated a motor tract initially, and then advanced into the delayed centre, as shown by the appearance of the subsequent hump.
(D) Record shows responses to stimulation with electrode inserted in different portions of the hyponeural ganglion. At the second and third stimulation marks only a delayed response was found, as shown by the slow rising tension curves.


t
d
A
C
1 2 3 4 5 6
B
1 2 3 4 5 6 7 8 9 10 11 12 13
D
1 2 3 4 5
1
1min

suction electrodes on the retractor muscle, electrical activity could be recorded from this muscle.

The muscle cells of the retractor muscle are long (often over one centimetre) but have a small diameter, 8 to 12 microns. For this reason many fibres at once are sucked into the tip of the electrode and due to the need to use a fairly large size of electrode this is unavoidable. Kymograph recordings have shown that there is much spontaneous activity in the muscles under normal conditions though only rarely does this movement show a predictable pattern. For this reason the electrical activity recorded using suction electrodes is normally from a large number of units all seemingly firing at random and fairly frequently. Much of this electrical activity is due to the initial disturbance of the nerves and muscles setting up the preparation and after about fifteen minutes the electrical activity subsides and just a few units fire at intervals, Fig. 6.

To show that the electrical activity recorded was not an artifact due to movement of the muscle, a number of controls were made. These involved using de-innervated muscle moved mechanically under different recording conditions and showed clearly that the activity recorded from intact muscle was not a movement artifact.

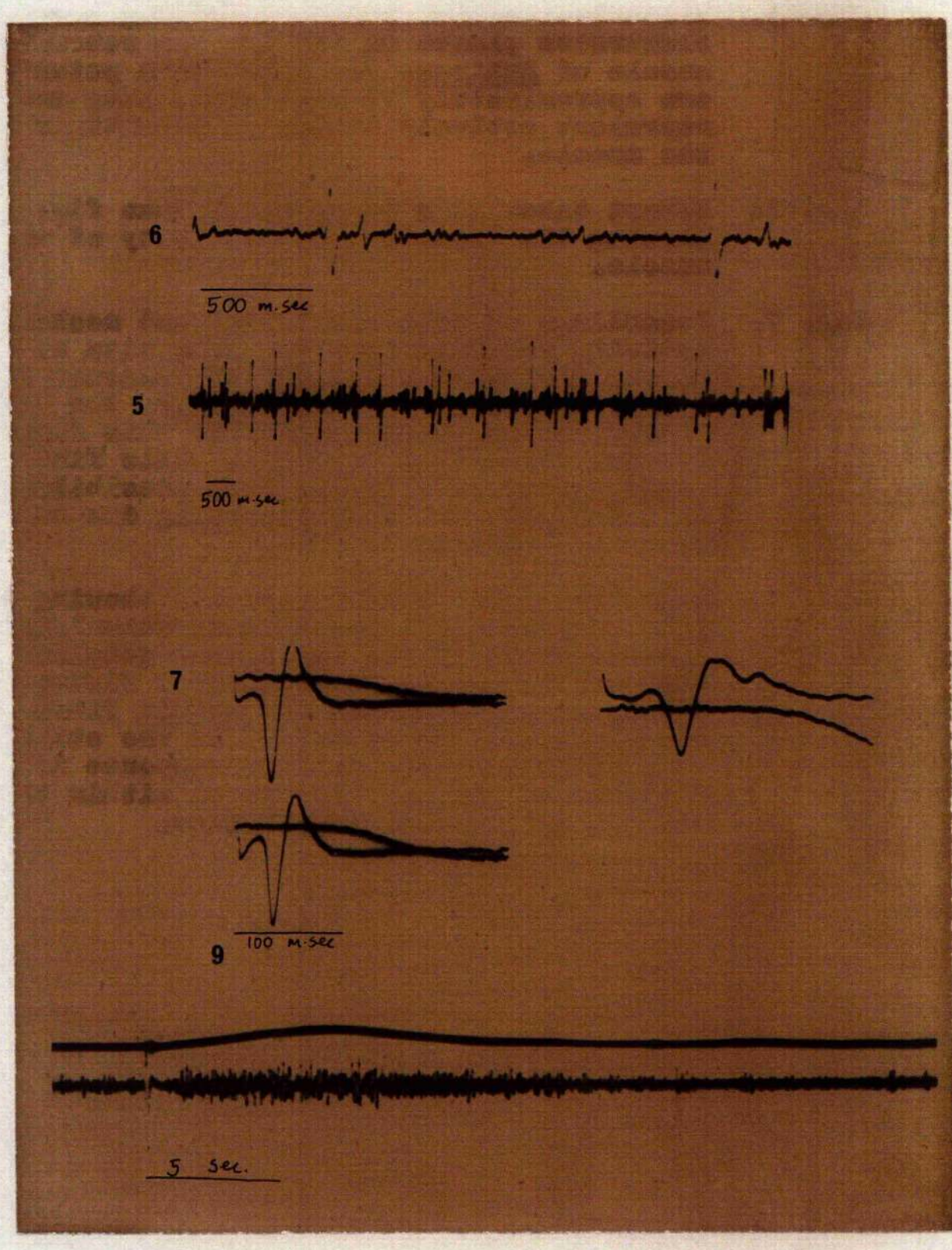
Fig. 5 filmed at a slightly higher film speed shows the

Fig. 5. Electrical activity recorded using suck electrodes placed on the lantern retractor muscle of Echinus. The spike-like potentials are approximately 75 millisecs. long and represent actively conducted potentials in the muscle.

Fig. 6. Record taken at a lower speed than fig. 5 showing the 'spontaneous' activity of the muscle.

Fig. 7. Recordings of both electrical and mechanical activity recorded from the same site on the muscle fibre. The latency of response is approximately 50 milliseconds for the electrical response and double this figure for the mechanical response. This finding would appear to eliminate the possibility of the electrical response being due to a mechanical artifact.

Fig. 9. Recording taken over 15 seconds showing the delayed mechanical response to stimulation of certain areas of the hyponeural ganglion. This response is due to the continued firing of a number of units within the muscle fibre many seconds after the cessation of the stimulus. This shows that the delayed response is due to features of the nervous system within the ganglion and not due to dual innervation.


6
500 m.sec
5
500 m.sec
7
9
100 m.sec
5 sec.

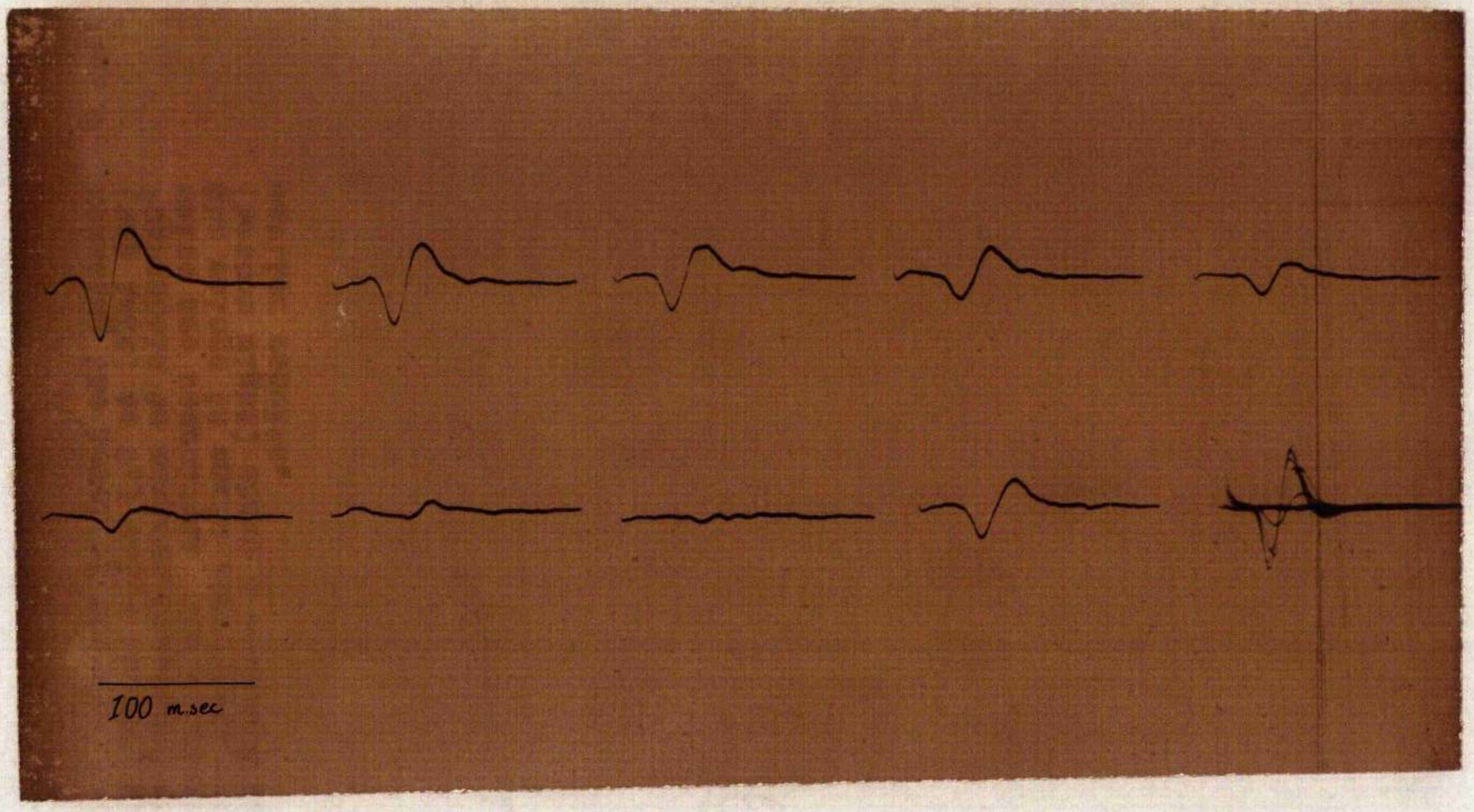
shape of individual electrical events. These electrical events apparently represent muscle spike potentials. The amplitude of the spikes depends very much on the recording conditions but the largest were about 80 to 90 microvolts. The duration of the biphasic spike averaged 75 milliseconds. It seems likely that the smallest spike potentials come from those fibres only partially sucked into the electrode and the larger spikes from those muscle fibrils most completely sucked into the electrode.

The electrical activity recorded is increased on stimulating the radial cord mechanically as would be expected from the results obtained using a kymograph to record the muscle contraction to similar stimulation. The final experiments in this series were carried out using stimulating electrodes placed in the hyponeural ganglion. At the same time as electrical recordings were made from the muscle a lever connected to an electro-mechanical transducer was used to monitor the movements of the muscle. Fig. 7 shows the electrical and mechanical activity recorded from the retractor muscle to stimulus of the hyponeural ganglion. The mechanical activity and the electrical activity were both recorded from the same point and it can be seen that the mechanical response has approximately twice the latency of the electrical activity.

Fig. 8 again shows the response to electrical stimulation of the hyponeural ganglion. The threshold for stimulus to be effective is 0.5 volts as a single pulse for 1 millisecond. At this level of stimulation single units can be seen to fire occasionally. As the stimulus is increased the electrical response becomes a compound potential of many units and at about 7 volts the response reaches a maximum. The response to varying the pulse length shows a similar 'threshold to maximum' response. Experiments recording contractions of the muscle to stimulation of the hyponeural ganglion showed that a dual response was present (see page 17). This response was considered to be due to nervous interaction within the ganglion rather than dual innervation (Cobb 1966). Fig. 9 shows the electrical and mechanical activity of both types of response. The delayed response shown in figure 9 demonstrates that the delayed mechanical response is due to the same type of unit as is present during spontaneous movements and to the quick response in figure 5.

Moving the electrode progressively more distal to the area of hyponeural ganglion stimulated produced two effects. First, there was a marked increase in the latency of the response, and second, the duration of the compound potential was increased. In later experiments two electrodes were used to record from different sites on the muscle simultaneously. These experiments

Fig. 8. Reducing the level of stimulus from 7 volts (top left) to 0.3 volts (bottom centre) shows a 'threshold to maximum response' as more and more units are recruited. The response is repeated if the volage is again increased. The record (bottom right) shows a compound picture of the earlier results.

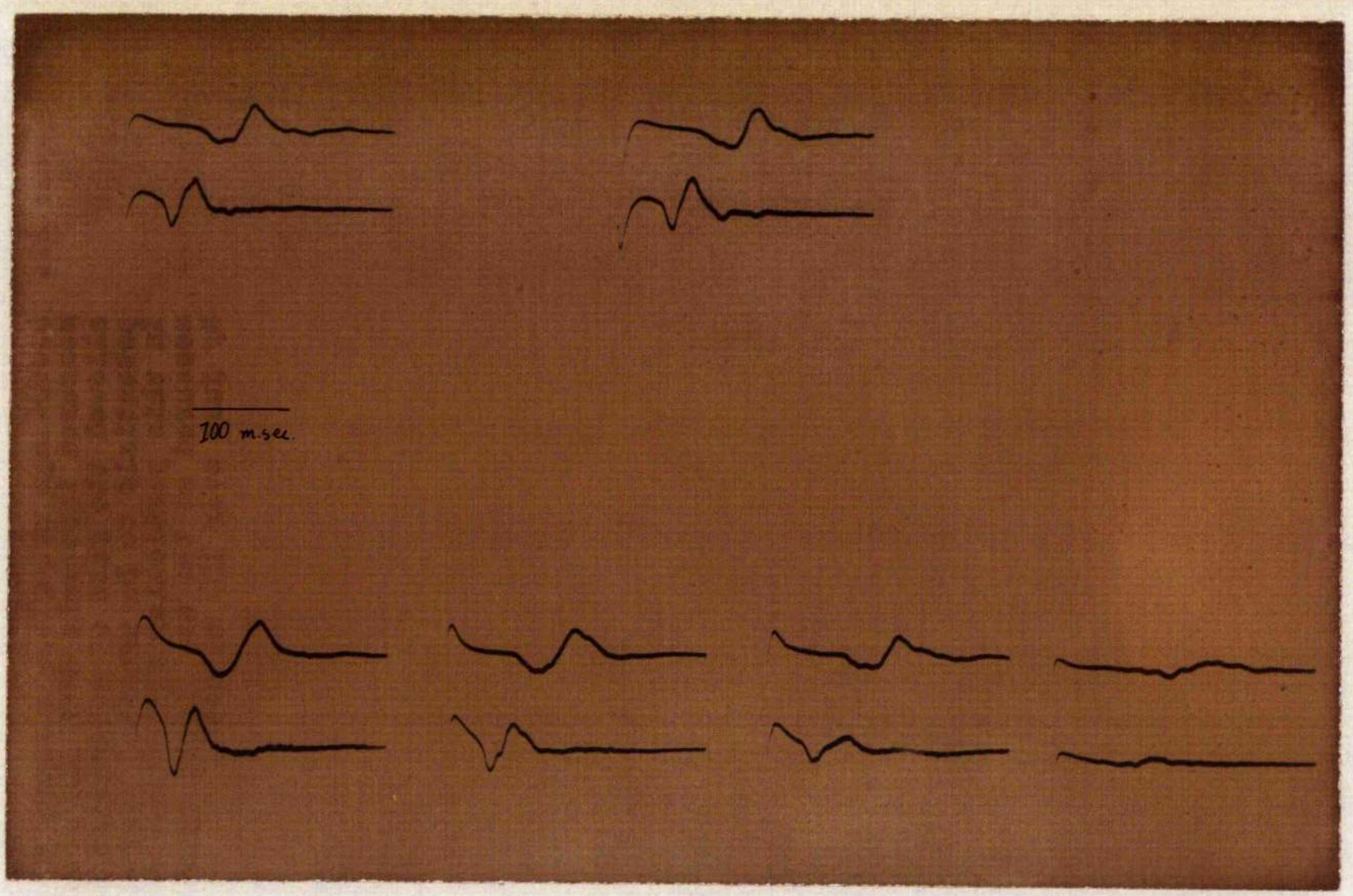

100 m.sec

(fig. 10) enabled the rate of conduction of the electrical activity down the muscle to be measured. The increases in the latency and duration of the compound potential are clearly observed using two electrodes and it can be shown that the threshold of stimulation that is effective is the same throughout the whole muscle.

Recordings using intra-cellular electrodes.

Intracellular electrodes were used to penetrate the muscle cells on several occasions. It proved very difficult to penetrate the muscle cells and only on four occasions was this achieved. From these four penetrations the resting potential of the muscle cells was approximately 65 millivolts. No potentials were recorded from these muscles and on three occasions the electrode rapidly came out of the cell. Renewed attempts to obtain satisfactory results have been initiated.

Fig. 10. Recordings using two electrodes placed at measured intervals down the muscle fibre allowed the rate of conduction of the action potentials to be measured approximately. In this particular set of records the latencies measured at the two recording sites differed by just over 100 m. secs.


100 m.sec.

4. Electron microscopy. Three methods of fixation have been applied.

(a) Osmic acid (O_sO_4). Tissue was left intact on the pyramids of the lantern, and as much as possible of the material surplus to the investigation was removed, by dissection. Fixation in situ was carried out since fixed tissue proved easier to handle and orientate than tissue dissected live. Tissue was immersed in 1% osmic acid in sea water for periods of ½ to 1½ h (usually 1 h). Early material was fixed in OsO_4 buffered to pH 7 with veronal - but later buffer was omitted without affecting fixation. Dehydration was in acetone, and embedding was carried out in Araldite.

(b) Glutaraldehyde (glut/OsO_4). Tissue in situ was placed in 5% glutaraldehyde. (25% stock glutaraldehyde diluted to 12½% with distilled water and to 5% with sea water). Final fixative was buffered to pH 6.5 with sodium orthophosphate. Material was fixed for 4 h in glutaraldehyde, washed in running sea water for ½ h or longer, treated with 1% osmic acid for an hour and then embedded as above after dehydration.

(c) Osmic phosphotungstic (PTA). A 1% solution of osmic acid in sea water buffered to pH 7.0 (with veronal) was applied for 3 h with the tissue in situ. The method of Gray & Young (1964) was followed subsequently.

Ultra thin sections for electron microscopy were

MATERIALS AND METHODS.

The animals were obtained and kept as described previously. Four main methods of attack were utilized.

1. Intra vital staining with methylene blue. This method was used to follow the gross patterns of innervation of the lantern from their origin at the hyponeural tissue to the various component muscles. The technique also gave information on the nature of the hyponeural ganglion itself and innervation of the retractor muscles. It is not in general a very satisfactory stain by reason of interference of massive amounts of connective tissue.

2. Silver staining. The standard Holme's and Golgi-Cox methods for nervous systems were applied to this echinoderm material without success. The Holmes's method did stain the nerve tracts in both muscles and hyponeural areas, but failed to differentiate between these tracts and the surrounding connective tissue.

3. Sundry other staining methods were utilized. The use of Heidenhain's azan (Pantin 1946) revealed the course of numerous nerve tracts, the identity of many of which have since been confirmed by the use of the electron-microscope. Information on the hyponeural tissue was expanded by thin sections of Araldite-embedded material subsequently stained by toluidine blue.

prepared on a Porter-Blum microtome using glass knives. They were mounted usually on unfilmed grids, though some sections were placed on filmed grids. Examination was with a Siemens Elmiskop 1 at 60 and 80 kV.

Astropecten irregularis

Specimens of Astropecten irregularis were supplied by the Scottish Marine Biological Association at Millport, Isle of Cumbrae. Three methods were used to study the histology and morphology of the ampullae of the feet. Firstly, vital staining with methylene blue by the method of Smith (1950) yielded results comparable to his. Secondly, paraffin sections of the whole arm stained with Heidenhain's azan provided details of the gross anatomy. Care is essential however, because in Astropecten fine fibred muscle and connective tissue of types absent from Echinus appear similar to nervous material when stained.

Thirdly, material for electron microscopy was fixed in unbuffered osmium tetroxide (1% in sea water) for periods between ½ and 1½ hrs. The material was fixed in situ to ensure exact orientation and to reduce the risk of contamination by spicules of calcium carbonate. Material was then dissected from the ossicles of the arm in 20% acetone, dehydrated in acetone and embedded in Araldite. Silver sections cut sequentially and occasionally serially in known planes were mounted on grids without support films and stained

for 3 min. in lead citrate followed by 3 min. in 2% uranyl acetate. The lead citrate was prepared by placing 0.04 g. of lead citrate in 10 ml of double-distilled water and adding 0.5 ml 10 N sodium hydroxide. This solution was made up fresh each time it was required. Material prepared with glutaraldehyde/osmium tetroxide and osmium tetroxide/phosphotungstic acid, as described by Cobb & Laverack (1966b), was used to study the synaptic regions. Whole tridentate pedicellariae from the test of Echinus esculentus were fixed in 1% osmium tetroxide made up in sea-water buffered to pH 7.5 with veronal acetate. After fixation for 45 min. material was dehydrated in a graded series of ethanol to absolute ethanol. Some of the material was then stained for 3 hours in 1% phosphotungstic acid in ethanol, the remainder was not treated in any way. Material was finally embedded in Araldite. In some cases material was dissected free from the ossicles after fixation then dehydrated with ethanol, stained and embedded.

While the work was in progress it became clear that the calcareous ossicles of the pedicellariae interfered with the preparation of ultra-thin sections and thus decalcification would be required if a full examination was to be made. A series of experimental fixation procedures were made and the results compared with muscle dissected free from the ossicles and fixed as described above. The most satisfactory results were obtained using material fixed in unbuffered osmium

tetroxide in sea-water for 1½ hours followed by four days in a saturated solution in water of diamine-ethane-tetra-acetic acid (Versene). Material was then dehydrated in acetone and embedded. Phosphotungstic acid stain was not used on decalcified material.

Decalcified material when examined with the electron microscope at high resolution showed that the unit membranes of the cells were less well defined than in untreated material, but gross morphological details appeared unaffected.

Ultra-thin sections, supported on unfilmed grids, were stained for 3 minutes in lead citrate followed by 3 minutes in 2% uranyl acetate. The lead citrate was prepared as described previously.

RESULTS.

Anatomical Studies.

In this study of echinoderm neuromuscular systems several entirely different preparations have been used. Two of the main preparations were derived from the Echinoid Echinus esculentus and one from the Asteroid Astropecten irregularis. The gross anatomy and fine structure of these three preparations is described below.

The lantern retractor muscle.

Physiological and fine structure studies were carried out on the lantern retractor muscle of Echinus.

As is well known from the earlier descriptions, Aristotle's lantern comprises five sets of complex calcareous

ossicles and associated muscles. The salient features are the five jaws, the pyramids, each of which carries a single tooth. Attached aborally to the pyramids along the epiphyses are the lantern protractor muscles which extend to the interradial positions between the auricles of the perignathic girdle. External to the pyramids in the oral position are the retractor muscles that are inserted distally on the top of the auricles. A third set of muscles, the commissator muscles, join the five pyramids into a pentagon. The compass elevator muscles are placed aborally where the gut extends from the lantern into the coelomic cavity (see Cuenot 1948).

The lantern protractor muscles extend from the pyramids to the auricles, and also along the broad faces of the pyramids towards the insertion of the retractor muscles. This sheet of muscle covering the sides of the pyramids was first described in Cobb (1964). It is proposed that it should be considered as a separate entity to the main bulk of the protractors since the characteristic innervation derives from a different point of origin at the hyponeural ganglion (Cobb, & Lavarack 1966a) and is quite separate from that of the well known protractor muscles. Nothing is known of the embryonic development of this system so confirmatory evidence is lacking. These muscles then are in continuity with the protractors, and also with the retractors, to which they are attached by a sheet of connective tissue. They are inserted on the pyramid in a groove that extends from the end of the protractor

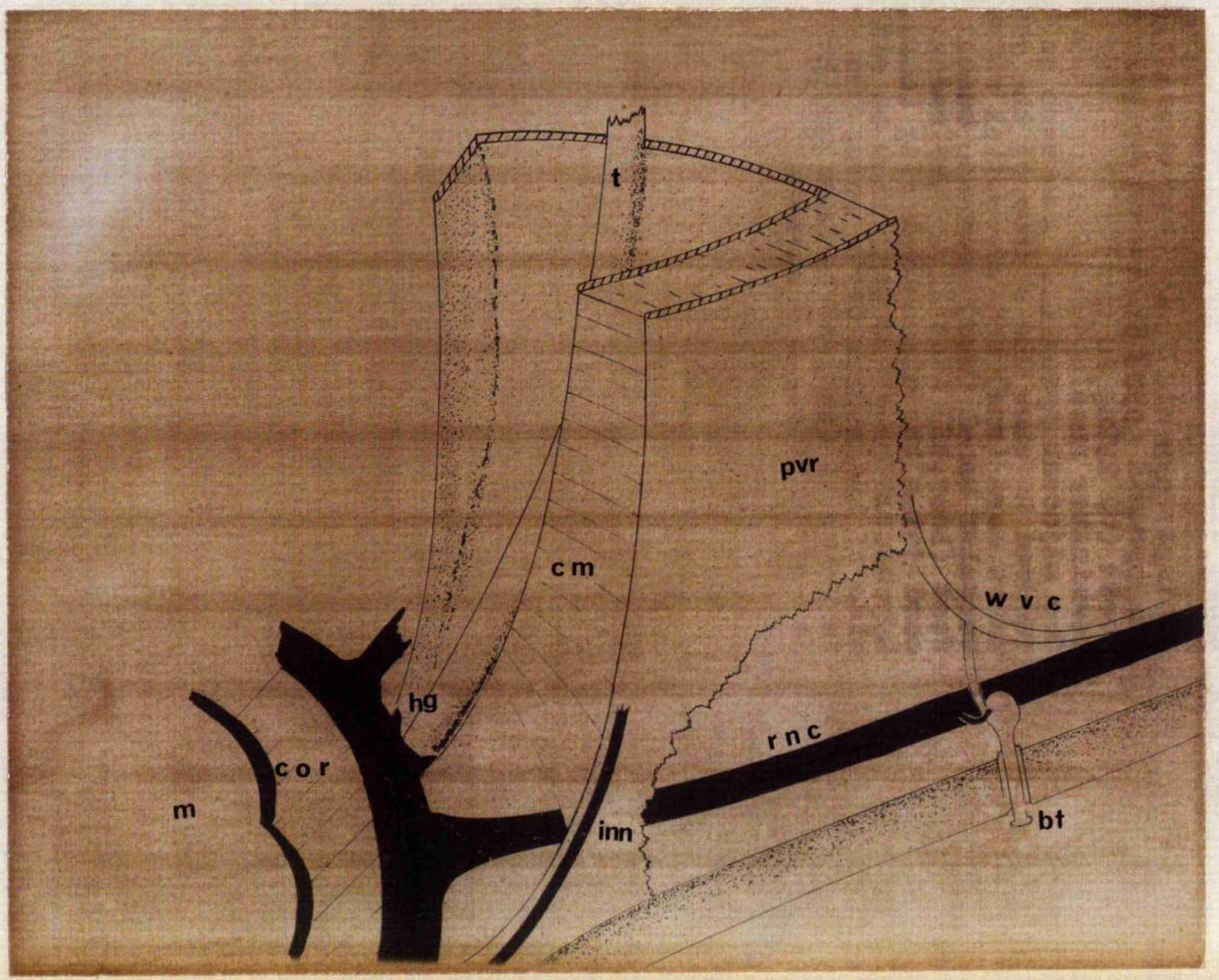
attachment to the same circular depression that marks the attachment of the retractors on the lantern. These sheets of muscles are tentatively named the postural muscles since contraction leads to a change in the attitude of the pyramid relative to the fixed point of the auricle.

The nerve tracts that serve the muscles of the lantern have been mentioned by Chadwick (1900). This description can now be modified and expanded. Chadwick described five radial plaques of hyponeural tissue at each radius. Close examination reveals that there are ten separate areas of hyponeural tissue. These lie one on each side of each radial nerve cord just above the circumoral ring in the hollow formed by the serrated edge of the pyramid and the tooth (figure 11). A pair of nerves arises from each hyponeural ganglion. These hyponeurally derived nerve tracts have since been shown to branch further (Holtt & Ewer 1963).

The use of numerous specimens stained with rongalit methylene blue has made it possible to trace rather more of the innervation. Figures 12A and B indicate the distribution of nerves after they originate at the hyponeural ganglion.

Two main nerve branches originate at each hyponeural site. The first of these descends around the base of the tooth and there divides with one branch passing up into the lumen of the pyramid, behind the tooth, and extending to innervate the compass muscles at the apex of the lantern; the other branch continues around the base of the retractor muscle, with

Fig. 11. Three dimensional reconstruction to show relationships of nerves associated with a single pyramid from Aristotle's lantern. The pyramid (pyr) is opened to show the tooth (t) lying in the centre. The comminator muscle (cm) form a sheet between two adjacent pyramids. The test is removed to show the course of the radial nerve cord (rnc) and the radial water vascular canal (wvc) from the buccal tube foot (bucc.tf) distally. The radial nerve cord joins the circumoral ring (cor). The hyponeural ganglion (hg) lies on either side of the radial nerve cord. The innervation (inn) of lantern muscles arises at the ganglion. m = smooth.


t
pvr
cm
wvc
hg
rnc
cor
m
inn
bt

finer branches innervating this muscle, and then passes up the outer surface of the pyramid. A fan-like branching of fine nerves occurs on to the surface of the postural muscles.

The second hyponeural nerve divides almost immediately into two. Boitt & Ewer (1963) have shown the same situation in Parechinus. One tract ascends the inner serrated edge of the pyramid, innervating the comminator muscles as it goes, and about halfway up the pyramid divides again with one branch continuing to the comminator pyramid muscles and the other branch to run over the upper pyramid to the rotula muscles. The second tract passes up the hollow between the tooth and the inside surface of the pyramid. It then curves under the epiphysis to innervate the lantern protractor muscles. Further details on innervation will be found in Cobb & Laverack (1966b).

The tubefoot of Astropecten

The tube foot of Astropecten, as in other echinoderms, is a dilatation of the water vascular system. A branch from the radial vascular canal opens orally into a projecting tube foot and aborally into a bifid ampulla with a thickened seam on each side (fig. 13). The tube foot differs from that of most starfish in lacking a podial sucker, but this is of no significance here. Thickened distal and proximal bands of tissue, which Smith named the seams, extend in an oral direction down the neck of the ampulla and terminate in two medial and one lateral

Fig. 12. The innervation and location of the lantern musculature.
A. bisected pyramid viewed internally, ep = broken epiphysis; pyr = broken edge of pyramid Innervation is from the hyponeural ganglion (hg) by way of the protractor nerve trunk (pr.n), and the comminator and rotula nerves (crn), which divide into their respective branches distally.
B. A single pyramid viewed externally. The tooth (t) is set within the pyramid, and fine nerve trunks extend longitudinally on the surface. The tooth projects from the base of the pyramid (tt). The epiphysis (ep) joins the two sides of the pyramid aborally. The insertions of the muscles are shown along the sides of the pyramid; pr. m = protractor muscle, po.m = postural muslce, rm = retractor muscle. Their innervation is represented by n.tr the main nerve trunk originating at the hyponeural ganglion, po.n the nerve supply of the postural muscle, and pr.n the protractor nerves.

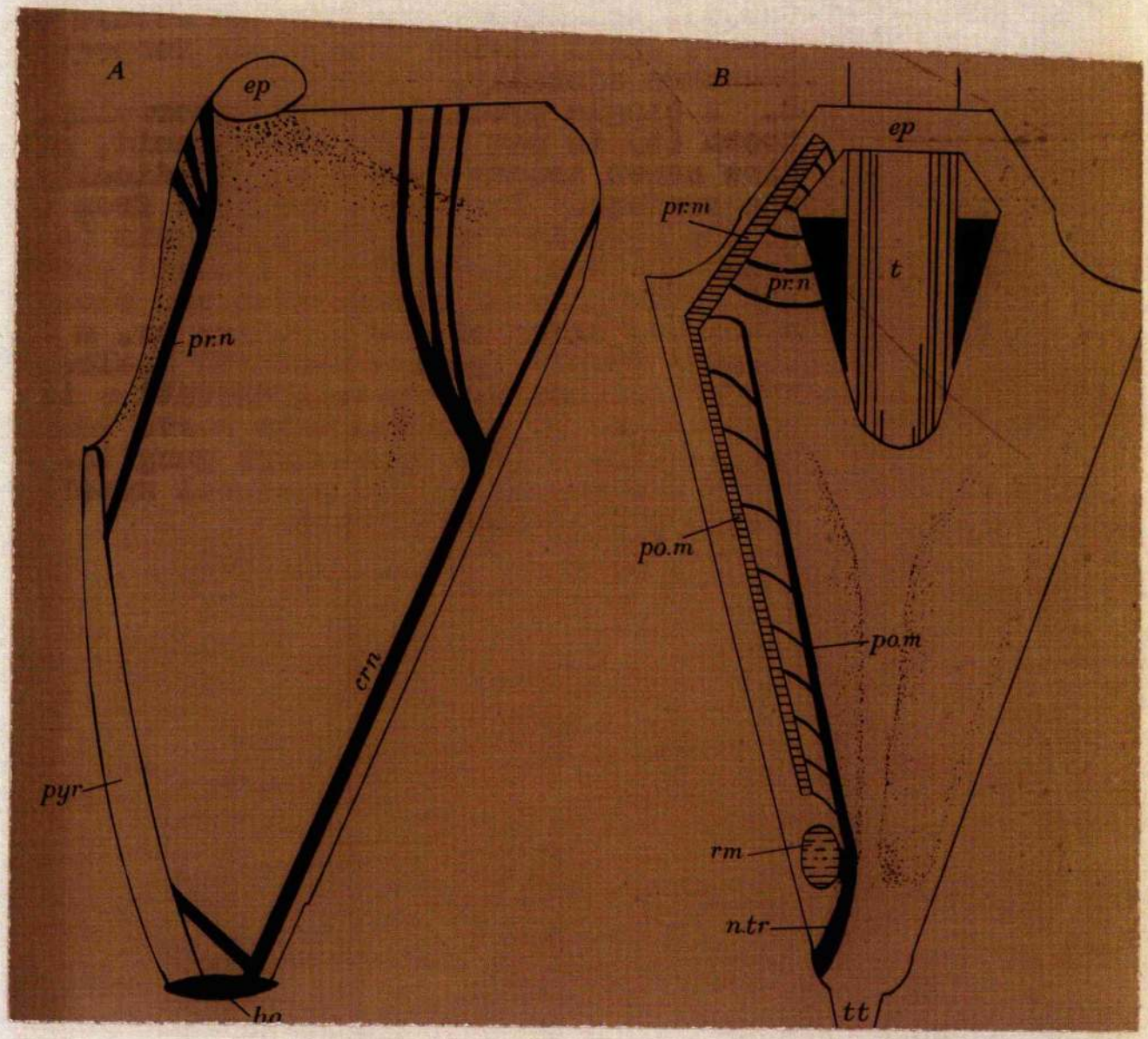
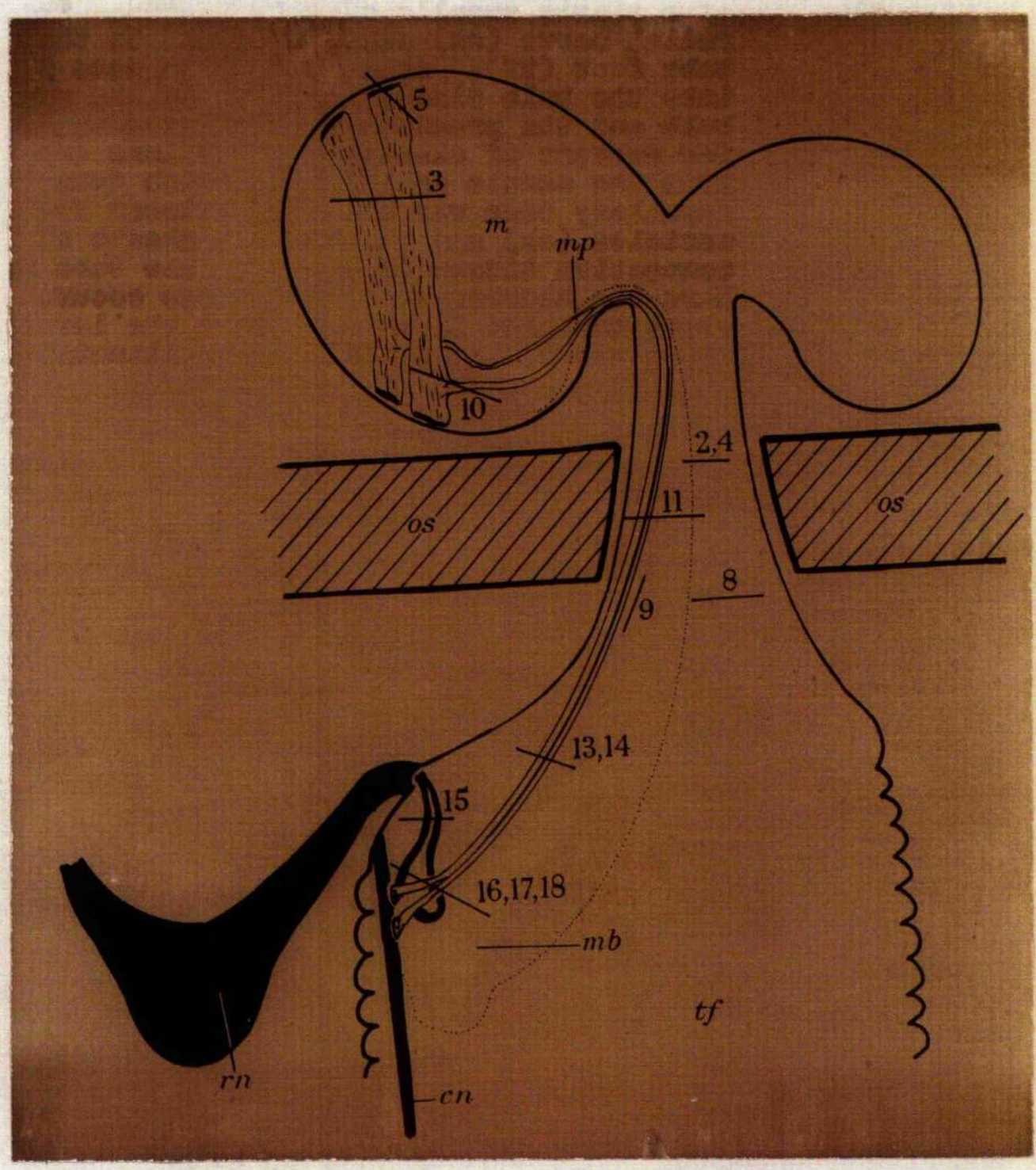

A
ep
pr.n
cr.n
pyr
ba
B
ep
pr.m
pr.n
t
po.m
po.m
rm
n.tr
tt

Fig. 13. The general layout of the innervation of a single ampulla of Astropecten. The radial nerve (rn) sends a branch to the tube foot (tf). Axons from this pass down into the bulb tissue (mb). Only the medial bulb and the proximal ampulla seam are shown for reasons of clarity. Extensions (mp) from the muscle cells (m) stretch down the ampullary seam via the neck between the ossicles (os) and attach to a sheath of connective tissue (cs) within the tube foot. Here the neuromuscular junctions occur. The short numbered lines represent the levels which the electron micrographs illustrate.


5
3
m
mp
10
2,4
os
os
11
8
9
13,14
15
16,17,18
mb
tf
rn
cn

bulb at the point so labelled in fig. 13. The proximal seam is larger than the distal one. The bulbs project into the lumen of the tube foot, and are attached to the thick collagenous wall which surrounds this structure. Long muscle fibres are over the bifid lobes of the ampulla and embed in the outer layer of connective tissue which encloses the ampullae as a whole. Methylene blue selectively stains long flattened entities in the muscle, and these follow the curve of the ampulla and agree with the description of the ribbon axons of Smith (1950). Previous experience with Echinus has indicated that the nervous tissue of echinoderms can often be distinguished by a diffuse pink colour when stained with Heidenhain's azan (Cobb and Laverack, 1966b). This colouration appears to arise from the fineness of the fibres rather than the staining properties of the nervous tissue. Care is essential however, because in Astropecten fine-fibred muscle and connective tissue of types absent in Echinus cannot be distinguished from nerves by this stain. The seams are clearly fibrous but whether of nerve, muscle or connective tissue could not be decided with azan. The nerve which branches from the radial cord to the tubefoot is composed of two parts, as seen in section, separated by a thin lamella of connective tissue. According to Smith (1950) the aboral section contains the central distributory neurones from the cord to the bulb. This point has not been confirmed but it

is presumed that these neurones are the ones which will be revealed as directly innervating the muscles of the ampulla. These details cannot be seen in material stained by ordinary methods because they are obscured by much fine fibred material.

The pedicellariae of Echinus.

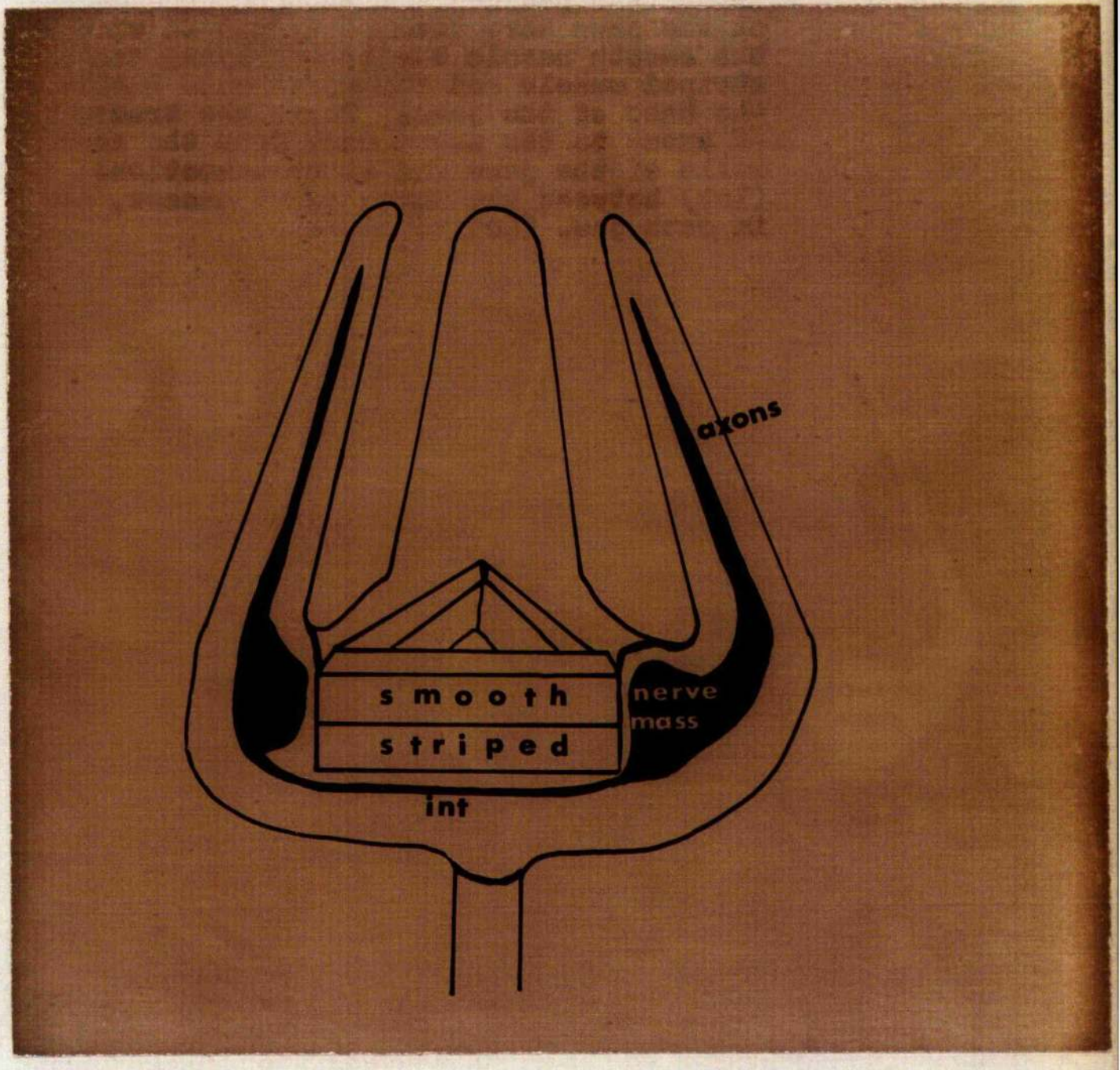
The tridentate pedicellariae occur on long bendable stalks externally all over the test. They range in size from 4 mm jaw length, at largest, downwards. The jaws are composed of a skeleton of ossicles formed from spicules of calcium carbonate. A number of muscle fibres join the three jaws to form a triangular shape, Fig. 14. At each apex of the triangle, the muscle fibres converge into a complex arrangement of ossicles that project out towards the mid-point of the triangle. The hard parts of the jaws articulate on basal plates formed from other ossicles. The bundles of muscles lie inside a wall of spicule containing epithelium that connects the three jaws laterally.

The muscle bundles of the jaw contain smooth fibrils overlying striped fibrils at the base of the jaws, the fibrils in both types of muscles are separated from their neighbours. There are about a thousand of each type of fibril and each fibril runs the full length of the muscle bundle from jaw to jaw.

Epithelium of pedicellariae.

Large cilia, averaging 30 u. in length, were clearly visible on the covering epithelium of the jaws of the

Fig. 14. Diagram showing the gross features of a tridentate pedicellaria of Echinus. Two of the jaws have been sectioned to show the smooth muscle bundle overlying the striped muscle and the nerve mass within the base of the jaws. There are tracts of axons to the nerve mass from the sensory cells of the jaws and interconnections (int) between the three nerve masses, one in each jaw. (Not to scale).


axons
smooth
striped
nerve
mass
int

pedicellariae when viewed with the phase contrast microscope. The cilia were separate and irregularly spaced in most areas but two tracts run down the sides of the inner faces of the tridentate jaws and there is a considerable aggregation of cilia in the sensory hillocks of the globiferous pedicellariae. There are fewer cilia on the outer faces of the jaws and the stem of the pedicellariae. The cilia are all motile, including those that are tightly packed together on the sensory hillocks. The beating of the cilia is irregular and unco-ordinated.

The Retractor muscle of Echinus.

Morphology and gross innervation.

The lantern retractor muscles are attached at the proximal end orally and externally to the pyramids of the lantern and at the distal end to the auricles.

Methylene blue intra vital staining of this region demonstrated the presence of elongated bodies somewhat resembling the ribbon axons of Smith, but serial sections routinely stained with Heidenhain's azan indicated no nervous structures save at the extremity of the muscle proximal to the pyramid. Nerve tracts at this site are plainly seen and easily distinguished from connective tissue by virtue of their characteristic staining properties. Clearly these are considerable concentrations of nerve fibres, and their apparent absence in more distal parts of the muscle might be due to the fine nature of the branches running through the muscle. In routine histological sections no structure was seen that could be correlated with ribbon axons.

The proximal area of the muscle in particular has been examined with the electron microscope to elucidate more details of innervation. It was already known from earlier studies (Laverack, unpublished) that nerve fibres were either absent or very sparse in the more distal regions of the muscle.

Attachment of muscles.

The proximal end of the retractor muscle is attached to the calcareous pyramid. Five types of tissue can be

distinguished at the attachment area. These are muscle, nerve fibres, collagen-like material, diffuse connective tissue and scattered coelomocytes.

Collagen-like fibres are not so closely packed as described for the hyponeural ganglion investment. It appears to play rather a supporting role in the area as a whole than as specific attachment for the muscle. The attachment of the muscle to the pyramid is mainly accomplished via diffuse connective tissue somewhat resembling the matrix of yellow elastic tissue in vertebrates. The proximal ends of the muscle fibres are extended in a series of finger-like processes that merge with the attaching substance (figure 15). Some muscle cells originate above the level of the pyramid and are not anchored to the attachment material. The whole structure is enveloped in cubical epithelium that conveys coherence to the retractor muscle.

At the attachment the muscle cells taper to a blunt point, but broaden out to 8 to 10 um diameter as they enter the main body of the muscle. There appear to be patent gaps between the cells at this point, but distally the fibres are tightly packed.

Muscle structure.

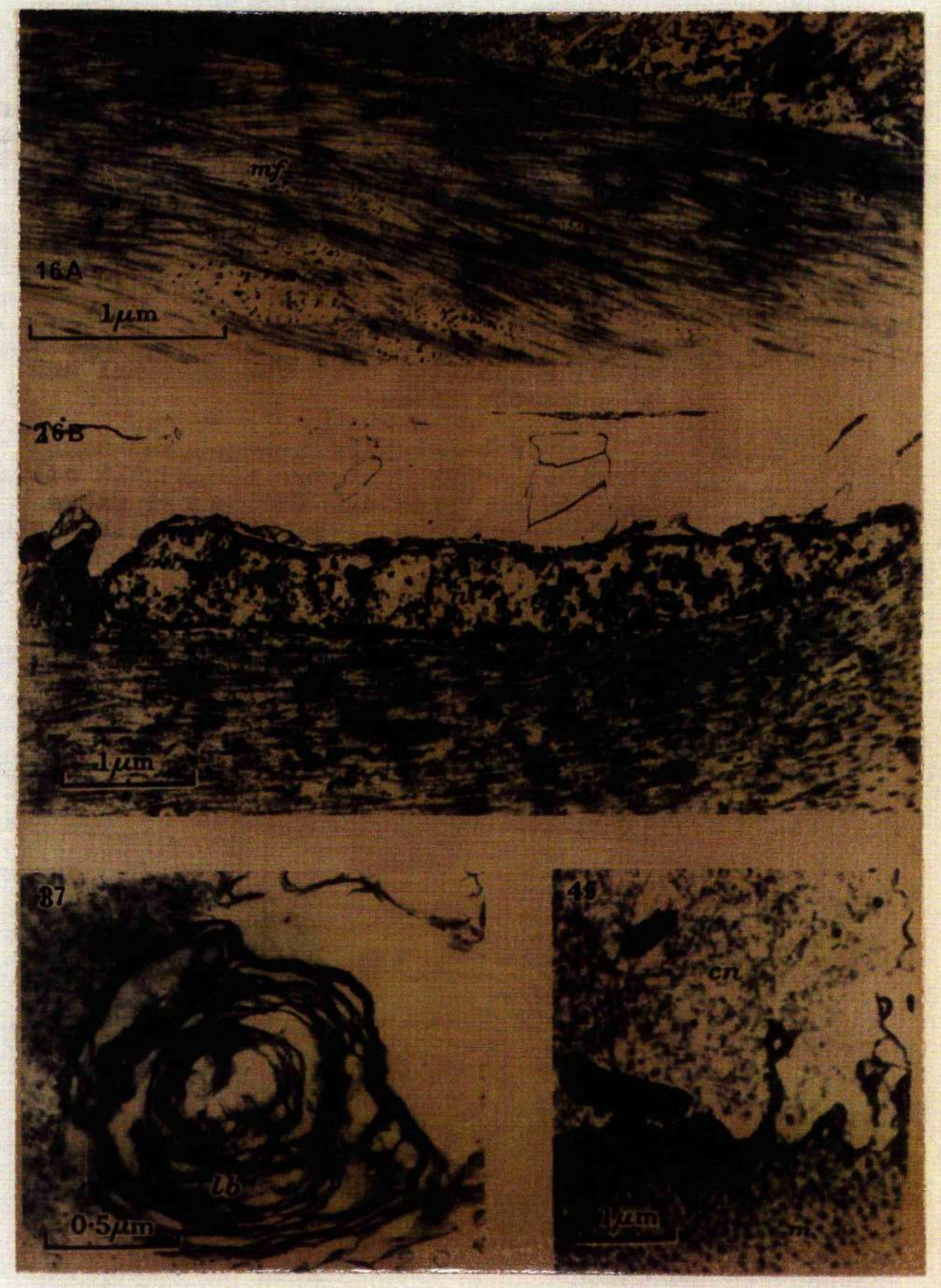
The lantern musculature is invested by a compartment of the water vascular system, comprising the lantern coelom. Each of the muscles is enveloped by a sheet of epithelial cells that mark off the muscular tissue from the coelomic

Fig. 16A Periphery of a muscle cell showing external coagulated material (cm) adhering to the surface, and the varied orientation of the myofilaments (mf) of the cell.

Fig. 16b The nucleus (n) lies on the periphery of the muscle cell, and is elongated in the direction of the long axis of the fibre.

Fig. 17 Lamellate body (lb) of unknown function, often associated with the proximal end of a muscle fibre.

Fig. 15 Matrix of connective tissue (cn) that provides attachment for the muscle cells (m) to the pyramid. The muscle fibre surface projects as small fing-like extensions into the connective material.


mf
16A
1μm
16B
1μm
87
lb
0·5μm
45
cn
1μm

cavity that surrounds it.

This epithelium is composed of cubical cells that are only loosely connected one to another. These cells are approximately 3 µm long by 2 µm deep, and are similar to those described below. A considerable part of the cell volume is occupied by the nucleus. The bases of the epithelial cells rest on a fibrous basement membrane or ground substance. The upper surface of the cells, which line the coelom, is covered with cilia. There is no apparent concentration of cilia into tracts, the whole coelomic surface of this epithelium being covered uniformly with the organelles. There is no unusual feature to be noted about these cilia whose structure is similar to that described by Gibbons (1961) for the motile cilia of Anodonta.

Muscle fibres.

The individual fibres that make up the lantern muscles are approximately 8 to 12 µm in diameter, and several millimetres in length (often extending the full length of the muscle, up to 2 cm). In parts they are often widely spaced from one another. This is probably the normal condition and not a contraction artifact.

The muscle fibres contain many short myofilaments, arranged longitudinally in the cell. These myofilaments average 1 to 2 µm in length, and 45 nm in diameter. Their axes are not strictly parallel with one another, but exhibit

a small disorientation to the long axis of the cell. In some cells it was noted that the filamentous areas were at a large angle to the long axis. In other cases it was seen that one cell contained filaments arranged in several different planes.

The nucleus of the muscle cell is small, and generally situated near the proximal end of the cell (see Figure 16). The amount of non-filamentous cytoplasm is also restricted. The cytoplasmic framework of the cell probably exists as a thin sheath around the myofilaments. Occasional islands of cytoplasm protrude between the myofilaments.

In the region of the nucleus the cell cytoplasm is often highly modified. The whole groundwork of the cell is permeated by endoplasmic reticulum, often closely associated with mitochondrial inclusions. There is often a close parallelism of the cell surface with the intracellular endoplasmic reticulum.

The cytoplasm of the cell is largely granular in appearance. Mitochondria are small, elongate structures with inconspicuous cristae. No Golgi bodies have been identified, but small (2 μm diameter) lamellate structures are present at the proximal region of each cell (figure 17).

The cells show conspicuous prominences of the surfaces. Between the cells there is a gap of about 250 to 400 nm, though this may often be considerably greater. The intercellular space contains granular debris, possibly representing

coagulated protein of the coelomic fluid that lies among the fibres. Each protrusion of the cell membrane into the intercell space is reflected by corresponding indentation of the surface of the neighbouring cell. No rigid cementation of one muscle fibre to another has been noted in these situations.

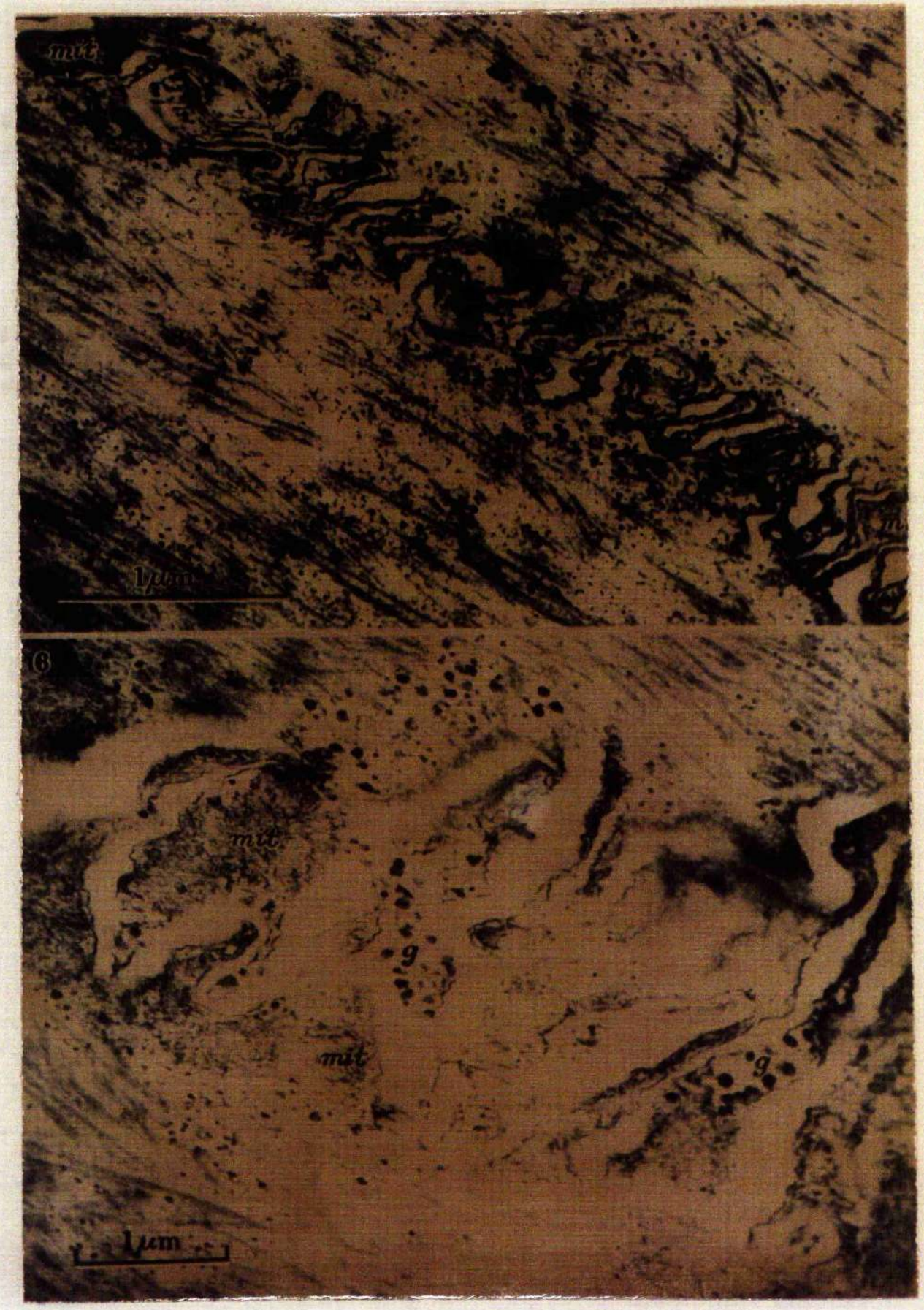
The relationship of one muscle fibre with its neighbours may thus at first sight appear to be a simple physical abutment that is readily separable upon contraction and relaxation. But in many areas there is a complex inter-digitation of the superficial prominences of the muscle cells (figure 18). Small finger-like projections, often bulbous at the end, lie in an alternating pattern along the length of the fibre. By this means the two adjacent cells are brought into contact along their common border. A second example is illustrated in figure 16, ~~plate 59.~~ In this case the processes are rather broader and more leaf-like.

These types of projection serve to bring the surfaces of the fibres into fairly close proximity. While this could be construed as a simple physical device to allow tension to develop and be transmitted within the fibres, it is remarkable that there is little sign of intercellular material to cement the junction more firmly. Rather the reliance is upon a peg and socket junction.

There is some modification of the inclusions of each muscle fibre in the region of the projections. The bulbous areas contain mitochondria with small closely packed cristae;

Fig. 18A Adjacent muscle cells show complex interdigitation of the cell walls with a patent cleft between them of approximately 100 nm. The projections are rather narrow, though some have bulbous ends. mit -mitochondria.

Fig. 18B Each cell prolongation contains a specialized entity, in the shape of modified mitochondria (mit) and a conglomeration of glycogen granules (g). The shape of the projections in this case is rather broad and leaf-like.


mit
1 µm
6
mit
g
mit
g
1 µm

associated with these are aggregations of droplets that resemble glycogen. These structures lie closely adjacent to the cell membrane.

Innervation.

As mentioned above, the attachment area of the retractor muscles is impregnated with nerve fibres. These can be traced to the origin of the muscle fibres and into the base of the muscle.

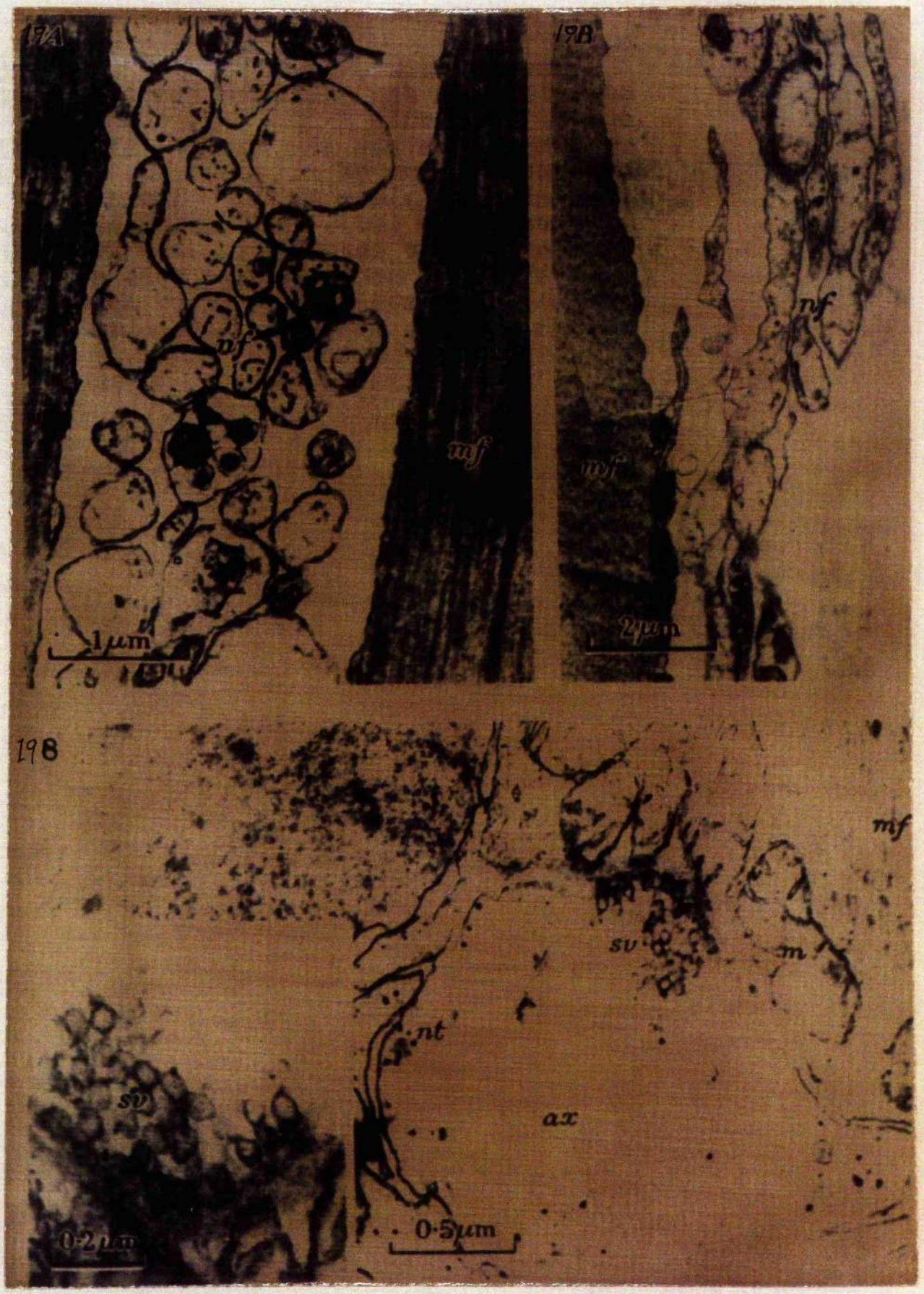
The attachment region contains bundles of axons (numbering between 10 to 100 per tract), averaging 0.75 µm in diameter. The majority (85%) of the nerve fibre population is of 0.8 µm diameter with the minority (15%) reaching some 0.25 µm. These small bundles of fibres branch from a main nerve trunk that contains about one thousand axons and originates at the hyponeural ganglion.

The smaller nerve tracts in the connective attachment split into further subdivisions that turn to run at right angles to the longitudinal axis of the muscle. These branches are shown in figure 19. The diversity of size can be seen.

In the muscle the nerve trunks have no enveloping membranes or glia, running only in spaces between the individual muscle fibres. As the muscle is penetrated at the proximal end the nerve bundles subdivide further until groups of 1, 2 or 3 axons only run together. Single nerve fibres

Fig. 19A The nerve fibres (nf) of the motor innervation to the retractor muscles run at right angles to the direction of alinement of the muscle fibres (mf). (A) fibres in transverse section, with myofilaments arranged longitudinally; (B) in longitudinal section, myofilaments cut across.

Fig. 19B A neuromuscular junction. An axon (ax) containing a few neurotubules (nt) lies against a muscle fibre (mf). In the region of contact the muscle fibre contains a concentration of mitochondria (m). The axon at this place shows a group of synaptic-type vesicles (sv). The inset shows this area at higher magnification. It is probable that the cell membrane has been cut somewhat tangentially and consequently appears diffuse, though such an appearance is also seen at synapses elsewhere.


19A
mf
1μm
19B
mf
mf
2μm
19B
mf
sv
m
nt
sv
ax
0·2μm
0·5μm

come into increasingly close proximity to the muscle cells, and are eventually enveloped in wing-like processes from the muscle cells.

Nerve fibres become fewer and fewer with increasing distance from the attachment at the pyramid. At 250 µm along the muscle they are already in short supply, above this level they are very scarce indeed. Some nerve fibres presumably do extend to these higher levels since some of the muscle cells of the retractor attach to the connective tissue between the protractor and retractors some millimetres above the pyramid.

On the basis of the present observations each muscle cell appears to be innervated by only one axon. At the level of the neuromuscular junctions the motor fibres are about 1 µm in diameter. Each muscle fibre is apparently innervated at only one site, and in the majority of cases this neuromuscular junction lies within 50 µm of the attachment zone. This would account for the sparsity of nerve fibres observed at more distal areas.

Neuromuscular junctions.

The neuromuscular junction of Echinus retractor muscle is a relatively unspecialized ending, and is difficult to characterize (as with synapses). Three criteria were applied to suspected neuromuscular junctions. Satisfaction of these criteria led to acceptance of the identification.

First, there must be close association of the nerve with the muscle cell such that the muscle cell often enveloped

the nerve termination in wing-like processes (figure 20).

Secondly, the muscle cell must contain mitochondria in considerable numbers immediately adjacent to the position of the nerve terminal (figures 19, 20, 21, plates 60, 61, 62).

Thirdly, the nerve fibre must contain an aggregation of small synaptic-type vesicles (figures 19, 20).

On the first count nerve fibres sometimes appear to run in a tubular extension of the muscle fibre. This is due to complete envelopment in a fold of the muscle fibre wall with meeting of the two component sides. The vesicular contents of the nerve ending are then occasionally seen alined relative to the processes rather than the main part of the myofibril. The presence and orientation of mitochondria are the most consistent indications of the neuromuscular junction. They only appear in any number in plate-like structures around the neuromuscular junction (figure 21). They form a band of organelles 2 or 3 deep and running for 3 or 4 μm along the myofibril. Mitochondria do occur elsewhere in muscle cells, but are much more scattered and smaller in size. The mitochondria associated with the neuromuscular junction are ½ to 1½ μm in length.

The membranes of the nerve and muscle cells do not appear modified, though some do seem to show the same diffuse nature as previously described synaptic membranes (figure 19).

Fig. 20. A neuromuscular junction. This example demonstrates the three characteristic features of such a junction; the synaptic vesicles (sv); the mitochondria (mit) in the muscle cell (m); and the wing-like processes (wp) of the muscle cell membrane enveloping the motor axon (ax.) Serial sections have shown that such areas are only a few microns in length.


sv
ax
wp
axil
m
1 μm
wp

Fig. 21. Three contiguous muscle fibres (m) cut at the proximal end. Two axons (ax) are enmeshed between the muscle fibres. This is probably an area close to a motor ending since the muscle fibres contain the usual aggregation of mitochondria (mit), and the axons show synaptic vesicle (sv).


m
mit
ax
sv
ax
mit
m
0·5 μm

The Ampullae of Astropecten.

The neck of the ampulla

Longitudinal and transverse sections were used to study the areas under investigation. The neck of the ampulla outside the region of the seams, is composed of 4 layers of tissue (see Fig. 22). The outer surface of the neck of the ampulla consists of a dense layer of collagenous fibres which are embedded in a matrix of connective tissue in total thickness up to 10 μ. The second of the 4 layers is muscular, with cells of various shapes closely packed with myofilaments. At this level there is no clear orientation into the muscle bands and tracts which are characteristic of the upper lobes of the ampulla (Fig. 23). In the neck region the myofilaments are arranged in various directions even within a single muscle cell. Such irregularity is a consistent feature of echinoderm muscle cells, (Bargmann & Behrens, 1963; Cobb & Laverack, 1966c).

The third layer is an ill-defined mass of muscle cells, many of which have large areas of clear cytoplasm with an irregular distribution of myofilaments. One even finds cells up to 20 u in diameter with no myofilaments anywhere in a particular cross section. In certain cells the myofilaments are arranged in the form of a spindle, the long axis of which may be in any direction with respect to the wall of the ampulla. They can hardly be considered as effective in contractions. Here again, even within a small

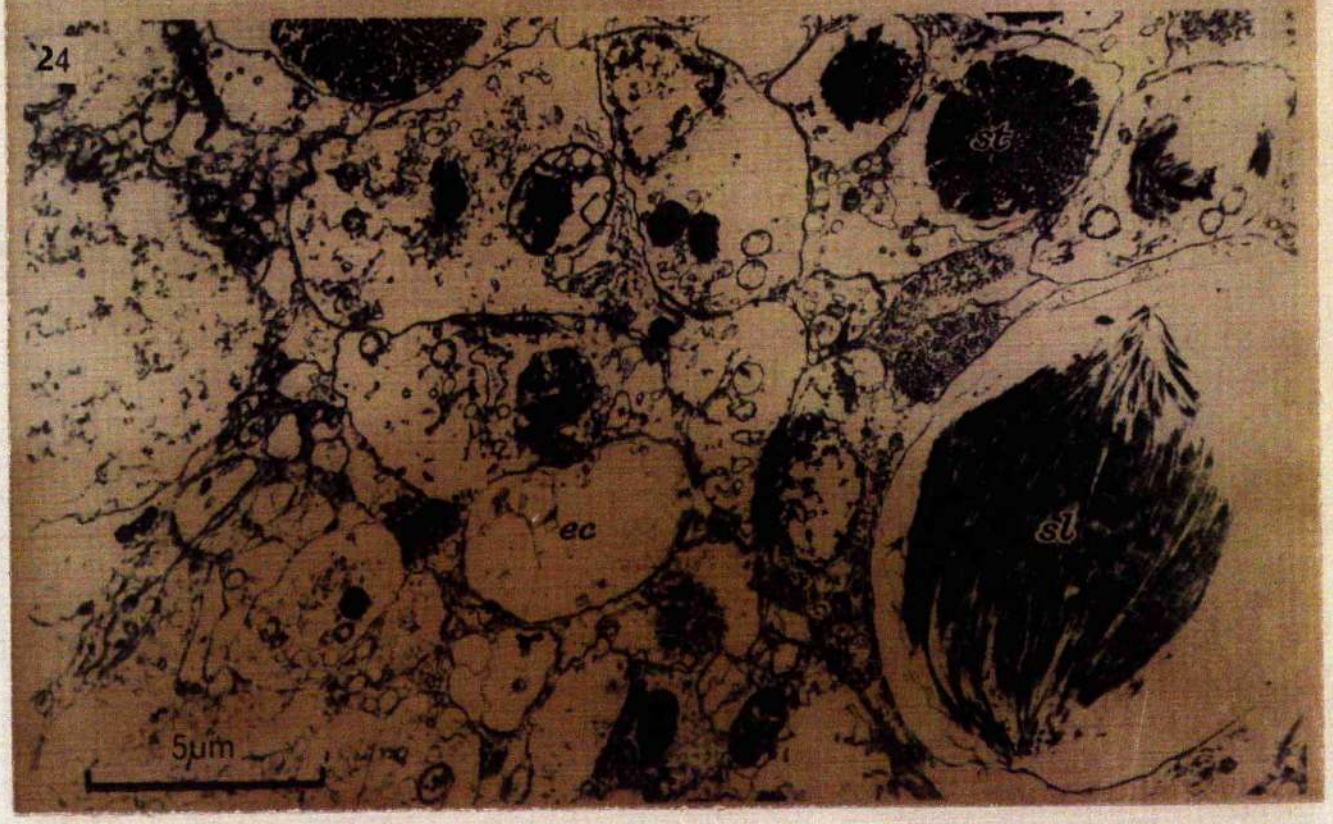
Fig. 22. The main tissues of part of a transverse section through the neck of the ampulla. The ampulla is surrounded by a dense collagenous connective tissue (cn). At positions proximal and distal to the central line of the arm there is a seam composed of extensions of the muscle cells (mp). Only one seam is shown. Attached to the connective tissue is a layer of normal muscle cells (m). Between these muscle cells and the inner lining of ciliated epithelial cells (ep) are other muscle cells with myofilaments in the form of a spindle, examples of which are seen here in transverse (st) or longitudinal section (sl). Other cells show areas of clear cytoplasm (cc).


mp
cc
cn
sl
m
ep
st

Fig. 23. Section through the lobe of an ampulla of Astropecten. Flat muscle cells (m) appear narrow or broad in section because they are cut in different planes.

Fig. 24. Transverse section through the neck of an ampulla. The spindle-shaped muscle cells are cut either in transverse (st) or longitudinal section (sl). There are large areas of unstructured cytoplasm in some of these cells, one of which appears completely empty (ec).


23
m
m
3µm


24
st
ec
sl
5µm

area, spindles of filaments, and even whole cells, exhibit a range of directions (Fig. 24). Moreover, the cells are intensely basiphilic in wax sections and in ultrathin sections stained with toluidine blue, thereby differing from the typical muscle cells of echinoderms.

The fourth layer of cells, lining the lumen of the neck, is a thin loosely knit sheet of ciliated epithelial cells. These are similar to the ciliated epithelial cells which are found covering the lantern of Echinus and within the ampulla of Asterias. Some of these cells have obvious vacuoles, as other authors have noted, but their function is not understood.

There is no structure in the neck or in the ampulla itself which can be identified as a nerve fibre under the electron microscope. Nerve fibres can be clearly identified elsewhere in echinoderms by neurotubules, dense-core vesicles, and by their general form and association with other cells.

Attachments of the muscles

The muscle cells of the ampulla are attached to the outer collagenous sheath (Figs. 25, 26), as already described in Asterias, but there are other types of attachment. A large class of specialized contacts can be recognized because thickenings occur at the plasma membrane within the cell where it comes against other structural material. The granular material of the thickening lies against the inner leaf of the unit membrane which is already thicker than the outer leaf.

Fig. 25. Attachment of the muscles (m) to the collagenous walls. Thickenings (at) occur inside the cell membranes at the point where muscle cells attach. Many collagenous fibres (about 100 nm thick) are present in the outer layer of connective tissue.

Fig. 26. Higher magnification of the attachment of figure 5, with thickenings inside the muscle cell membrane (cm) and the basal lamina (bl) of the non-cellular connective tissue (cn).

Fig. 27. Attachments between two epithelial cells (ep) similar to those between muscle cells. Arrowed: cell membranes at the outer boundaries of the thickenings in both cells. The inner leaf in each plasma membrane is thickened.

Fig. 28. Large muscle cell cut in tangential section, showing considerable areas of un-structured cytoplasm (cc) with few myofilaments (mf).


cu
m
at
1 µm
26
bl
cn
cm
0·2 µm
27
cp
0·1 µm
cp
28
mf
cc
2 µm

In muscle cells the myofilaments are tapered towards places where they insert into the granular thickening. Where cells attach to interstitial tissue or to non-cellular connective tissue containing collagen fibres (Fig. 26), the thickenings are present only within the attaching cell. If attachment occurs between 2 muscle cells, or 2 epithelial cells (Fig. 27) the characteristic thickening is symmetrical. Small strands of material are present between the unit membranes of 2 cells at points where these thickenings occur (Fig. 27 arrowed). Some cells show small wedges and spikes of tissue which interdigitate with those of an adjacent cell.

Seams of the ampulla

Two lines of tissue called seams lie along two opposite sides of the ampulla with one of them on the side nearest to the radial cord (Figs. 13, 22). The seam nearest the radial cord terminates at its oral end in two small bulbs of tissue which project into the cavity of the tube foot at about the point where it breaks when pulled off. The other seam runs to the lateral bulb. The bulbs were described by Smith (1930) from light microscope preparations under this name and can be seen in life. The seams are bordered by the layer of muscle cells containing a large proportion of clear cytoplasm (Fig. 28).

Under the electron microscope, the seams are revealed in longitudinal section as broad continuous tracts of narrow

elongated tubes running in an aboral-oral direction from the ampulla along the neck of the tube foot (Fig. 29). Most of these elongated tubes contain some myofilaments, and when followed in sequential sections they show themselves to be extensions of the main muscle fibres of the ampullary walls. Extensions of muscle fibres from all over the wall of the ampulla are collected into the seam (Fig. 30) and the extensions enter the seam throughout its course. The average diameter of each extension is about 2 μ, but within each seam they show a gradation from about 0.8 μ in diameter where they lie against the collagenous outer sheath to about 4 μ where they lie just beneath the epithelial inner lining of the lumen of the neck, (Fig. 31). Once they have been seen they can be immediately recognized by their regular pattern. Each extension of the muscle cells has a central core of myofilaments which stand out sharply from the surrounding clear cytoplasm. The myofilaments should be classified as thick since they measure between 20 and 25 mμ in diameter.

Around each myofilament a membrane combines with those around neighbouring filaments to form a delicate web (Fig. 32). The web perhaps supports thin filaments or represents thin filaments which have been changed during fixation, but in typical material it cannot be mistaken for alternative thick and thin filaments. However the web may be interpreted, it is unlike any structure in transverse sections of striped muscles or helical smooth muscles in that the fine elements

Fig. 29. Longitudinal section of the seam to show extensions of the muscle cells. When glancing sections of these extensions are cut, the cells (cc) may appear empty.

Fig. 30. Section through the periphery of the seam of an ampulla. An extension (mp) from the main soma of a muscle cell (m) enters the tract within the seam.

Fig. 31. Part of the seam of the ampulla, showing extensions of muscle cells in transverse section. A core of myofilaments within each process is surrounded by clear cytoplasm. The cell membranes of the extensions are more fluted than those of axons (e.g. figure 35, ~~xxxxxxxx~~) in the same animal, so that the extensions are distinguished from axons by their wavy outline in section.


29
cc
mp
mp
1µm
30
m
mp
2µm
31
2µm

do not share the same orientation as the thick filament. For the ampulla of Astropecten, there are no reports on the biochemical composition of the myofilaments to assist interpretation of the electron micrographs. Within the bulb at the base of the seams the core of myofilaments in the muscle extensions has a more granular appearance (Fig. 33) and thick filaments are not present. It is not clear whether this granular appearance is a continuation of the thick filaments or of the fine web of material, or of both. At points close to the attachment zones at their termination, the extensions of the muscle cells are narrower and the area of clear cytoplasmic is reduced in extent.

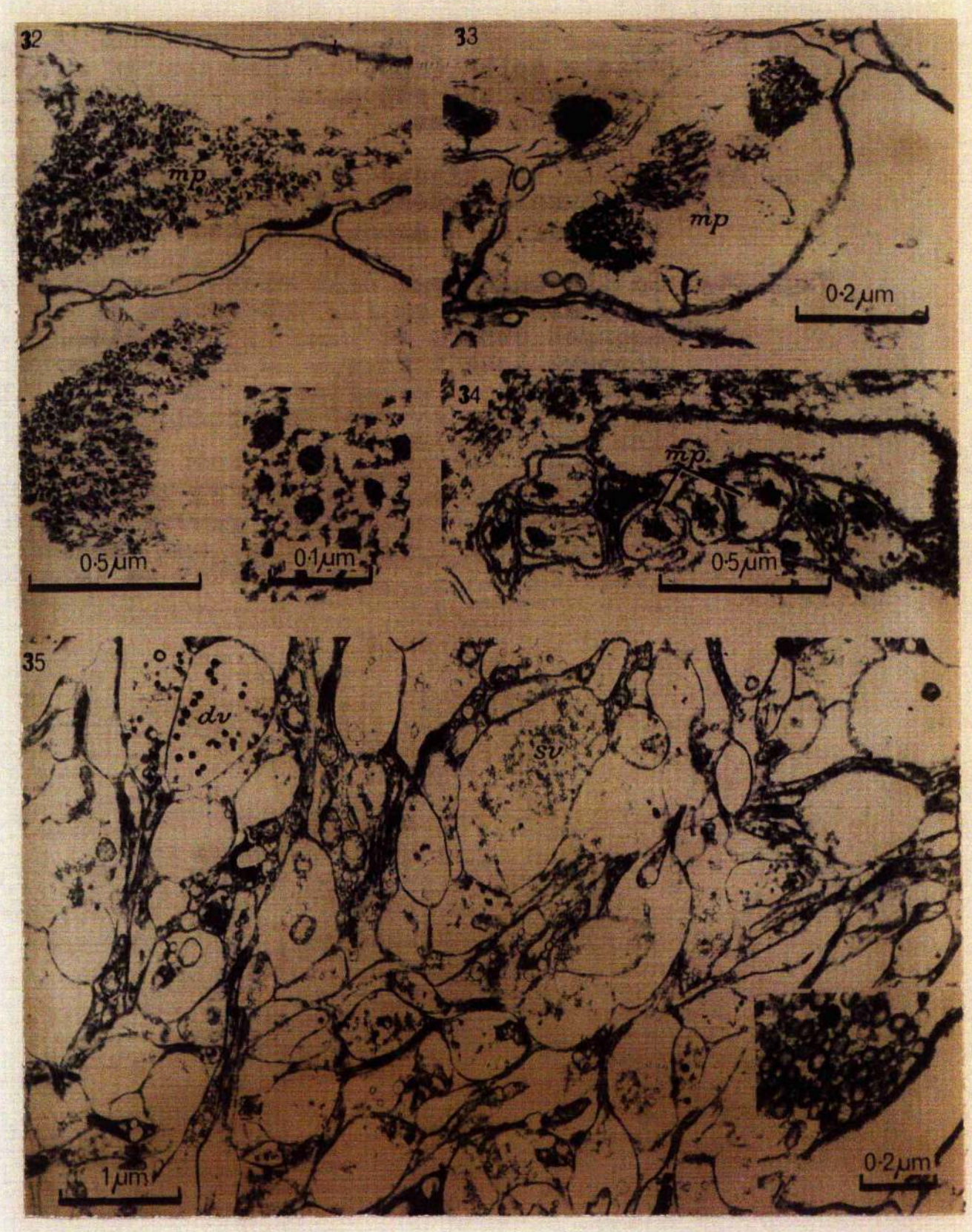
The distinction between muscle cell and nerve fibre is not at first apparent because myofilaments are completely lacking in some of the extensions of muscle cells in any plane of section. One can define a muscle cell as having myofibril somewhere within it. However extensions of muscle fibres without myofilaments can be distinguished by their fluted nature in cross section (Fig. 31) and the somewhat less dense appearance of the plasma membrane itself. The consistent orientation of the muscle extensions, with their tessellated appearance in transverse section in the upper regions of the seam, breaks down in the bulb, where a confused effect is caused by their interweaving among themselves. In this region some of the muscle extensions decrease to 0.2 μ in diameter,

Fig. 32. Higher magnification of extensions of the muscle cells (mp). A fine web of material surrounds the strongly basiphilic myofilaments, as in the inset.

Fig. 33. Within the tissue of the bulb, myofilaments in the muscle extensions (mp) eventually lose their identity in a granular mass.

Fig. 34. In the bulb some extensions of muscle cells (mp) are very small, and groups of them lie amongst others of more typical size. Some measure less than 0.2 um in diameter and the dense core of material appears to be composed of a delicate web of membranes that surround the myofilaments.

Fig. 35. Survey micrograph of part of the basal tissue of the bulb, showing interwoven axons which contain both synaptic (sv) and dense-cored vesicles (dv). Inset, structure interpreted as a synapse.


32
mp
0·5 μm
0·1 μm
33
mp
0·2 μm
34
mp
0·5 μm
35
dv
sv
1 μm
0·2 μm

about one tenth the original size (Fig. 34). This reduction in size may be caused by division.

The muscle extensions then pass through a mass of tissue which is clearly identified as nervous by its synaptic and dense-core vesicles and by neurotubules, and they terminate in attachments on the thick collagenous layer of the wall of the tube foot. It is in this area that specialized contacts occur between the muscle fibre extensions and the nerve fibres, and these contacts are therefore interpreted as the neuromuscular junctions.

Neuromuscular junctions

The general pattern of innervation is simple. A branch of the radial nerve cord extends to the tube foot, forms a collar around its neck, and branches to the bulbs. From this nerve some isolated neurons pass towards the periphery of the tube foot. Within the bulbs, nerves are largely limited to the lower portion, where contact is made with the extensions of the muscle fibres. The oral region of the bulb is composed almost entirely of axons (Fig. 35) whilst aboral to this is a region of intermingling axons and muscle extensions (Fig. 36).

The distribution of the neuromuscular junctions is also restricted. The processes of the muscle cells attach to the collagenous sheath of the tube foot, and neuromuscular junctions are found close to this attachment (Figs. 37-39).

Fig. 36. Survey micrograph of extensions of muscle cells (mp) which have invaded a region of axons (ax). It is within this area that the neuromuscular junctions occur.

Fig. 37. Processes of the muscle cells (mp) pass through the axonic material (ax) and attach to the sheath of connective tissue (cn).

Figures 38 and 39 Structures interpreted as neuromuscular junctions occur close to the site of attachment of the extensions of muscles upon the collagenous sheath within the tube foot. They are characterized by the close association of an axon (ax), a muscle extension (m) and the presence of synaptic vesicles within the axon. No membrane specializations have been observed.


36
mp
ax
mp
ax
2μm
37
mp
ax
0·5μm
cn
38
ax
m
0·2μm
39
m
ax
0·5μm

Each process appears to have one nerve ending upon it, but as it is not possible to trace individual muscle cells for long distances, a polyneuronal or multiterminal mode of innervation cannot be ruled out. No obvious specializations of the membranes are associated with the neuromuscular junctions, which are characterized by vesicles about 40 mμ in diameter within an axon where it is in apposition to a muscle cell. The morphology of neuromuscular junctions and axo-axonic synapses has received considerable attention in this preparatio and also in the lantern muscles and ganglia of Echinus (Cobb & Laverack, 1966 a, b).

Diverse techniques, including fixation with glutaraldehyde and osmic acid, separately or sequentially, followed by various staining techniques, including alcoholic phosphotungstic acid, have failed to demonstrate any specializations of pre or post-synaptic membrane at the junction. On the other hand synapses defined by vesicles have been observed many hundreds of times in consistent locations. They are constant in appearance but even if this evidence were not available, they are still the only contact between the nervous trunks and the effector organ. Synapses with a similar lack of specialisations form the only contact between nerves and ciliated cells which are controlled by nerves in the ctenophore Pleurobrachia (Horridge & Mackay, 1964).

The tract from the radial cord to the bulbs has two distinct regions separated by a collagenous sheath, but the

origins and separate destinations of these has not been followed. The axons which Smith (1950) described as running from the radial nerve to the bulbs of the tube feet were in the aboral part of this tract and were named the central distributory neurons. In the nervous region of the bulbs there is much interweaving of the fibres among themselves and here axo-axonic synapses are abundant (Fig. 35 inset). The presence of nerve cells in the base of the bulbs, and the large numbers of purely neural synapses, means that there is something more than a single system in which the central distributory axons form synapses directly upon the processes of the muscle cells. Probably a short interneuron which is intrinsic to the bulb is interpolated between them as Smith (1950) describes, or there may be a completely different system intermingled with the one described here.

Further details of the origin of the motor system in the radial cord and its distribution, is at present under investigation in both asteroids and echinoids. There are extensive areas of neuropile in the bulbs and in the nerves which branch from the radial cord. Co-ordination between different muscles of the ampulla may occur in this peripheral neuropile, with a similar morphological plan to that in the lantern of Echinus (Cobb & Laverack, 1966b).

It is clear that the topic would be illuminated if it were possible to make experiments which defined the physiolo-

gical pathways of excitation, and the relations between them, in the way which has been done for animals with nerve nets.

The pedicellariae of Echinus

The tridentate pedicellariae occur on long flexible stalks externally all over the test of Echinus. They range in size from a maximum of ¼ mm. jaw length, down to less than 1 mm. The jaws are composed of a skeleton of ossicles formed from spicules of calcium carbonate covered by a thin epithelium. A number of muscles join the three jaws and form a triangular shape in section (fig. 14). At each apex of the triangle, the muscle fibres converge upon a complex arrangement of ossicles that project out into the cavity formed by the three jaws. The hard parts of the jaws articulate on basal plates formed from other ossicles (see Hyman 1955). The muscles are protected by a wall of spicule-containing epithelium to the outside.

Structure of the muscles

The muscle bundles of the jaw contain two types of muscle fibre; smooth fibrils overlying striped fibrils nearer the base of the jaws (fig. 14). At the base of the jaws another set of smooth muscles function to open the jaws. These muscles are not described in detail. The fibrils in both types of muscles are separate from their neighbours. In each muscle there are about five hundred of both type of fibril and each component runs the full length of the muscle bundle from jaw to jaw. The striped muscle fibrils have an average diameter of 1.3 μ and the smooth muscle fibrils about 2.5 μ.

Each fibril has a single nucleus along its length, and this is usually situated towards one end of the fibril lying asymmetrically to one side of the fibril (fig. 40).

There are many mitochondria associated with the striped muscles. These are contained in projections of the cell membrane and form a ridge along the otherwise smooth outline of the fibril. The mitochondria average 0.2 u in diameter by 0.5 u in length. They occur less commonly in the smooth muscle fibrils.

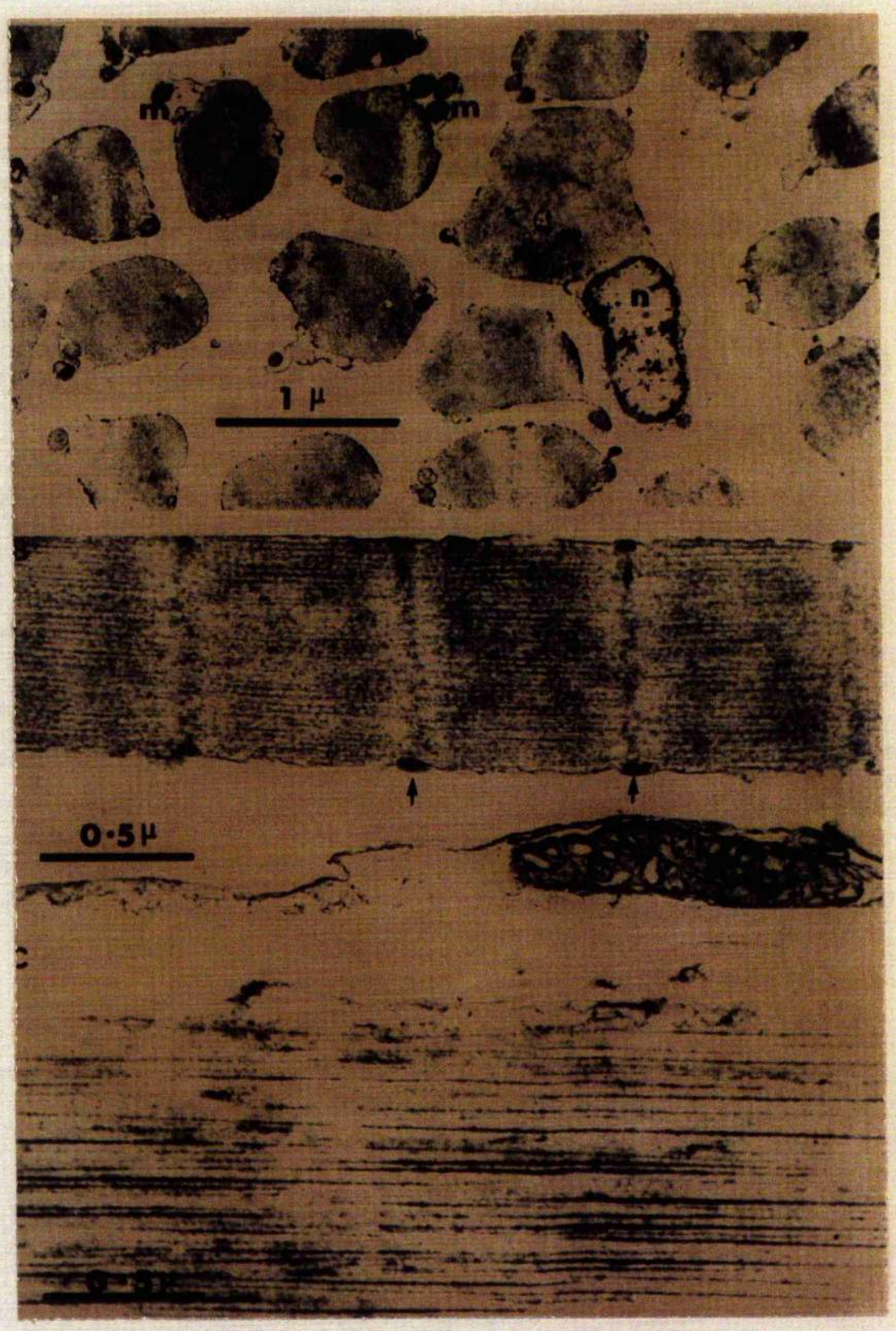
Striped and smooth muscle fibrils are shown in longitudinal section in Fig. 41 & 42. Each striped muscle fibril has a well developed T-system forming a tubular frame along its length. The fibril is encircled at each Z-band by a part of the T-system and approximately 15 cross connexions occur between Z-bands. The T-system can be seen in cross section at the Z-bands in the phosphotungstic acid stained material in Fig. 41. Fig. 43 shows a diagrammatic representation of a striped muscle fibril, illustrating the characteristic organization as described above.

In the relaxed state the Z-bands are separated by about 0.85 u and the filamentous arrangement is similar to that described in many other striped muscles. It does not, however, show well defined H- or M-bands. The thick myofilaments average 150 Å in diameter and the thin myofilaments average 50 Å. The striped muscle shows no regular sarco-

Fig. 40. Electron micrograph of striped muscle fibrils of the tridentate pedicellariae of Echinus in transverse section. Mitochondria (m) can be seen in projections of the cell membranes of the fibrils, as can a single nucleus (n).

Fig. 41. Longitudinal section of a striped muscle fibril to show the T-system. The T-system is cut in transverse section at the level of each Z-band (arrowed). The striped muscle shows no well-defined M-band and no trace of the thickening in the thick filaments forming the M-band.

Fig. 42. Longitudinal section of smooth muscle. The thick filaments all run parallel to the long axis of the fibril. Irregularly spaced thin filaments lie between the thick filaments. (P.T.A. stain).


m
m
n
1 μ
0·5 μ

plasmic reticulum, and therefore there is no diad or triad system formed with the T-system.

The smooth muscle which lies above the striped muscle is also composed of a collection of independent single fibrils. The myofilaments are orientated parallel to the longitudinal axis of the muscle and can sometimes be traced over 10-15 μ even in ultra-thin sections. The thick filaments are large with an average diameter of 400 Å, but show a considerable variation around this figure. The irregular thin filaments are 50 Å in diameter on average.

Attachment of the muscles to the ossicles of the jaws.

The method of attachment is similar in both smooth and striped muscles. At each end the fibrils break up into a number of finger-like projections, which may number 20, in a large smooth fibril.

The projections contain myofilaments, vesicles and mitochondria and where they abut onto the jaw ossicles they show thickenings within the plasma membrane. There are numerous fine fibres of collagen surrounding these projections. This type of skeletal-muscle linkage is similar to the attachment of the ampullary muscles to the podial wall demonstrated in Asterias (Bargmann and Behrens, 1963). Some projections from the fibrils characteristically differ from those described above. These do not contain myofilaments and pass through pores between the ossicles into a mass of nerve

fibres that fills the central cavity of each jaw. This nerve mass contains one or two thousand axons with occasional cell bodies. There are many axo-axonic synapses within the mass and the central area may probably be regarded as an area of neuropile. Within the axons, which vary in size between 0.1 μ and 2 μ, are concentrations of both synaptic-type and dense-cored vesicles. The nerve masses of each jaw are interconnected by small collateral nerve tracts.

Neuromuscular junctions

The area of attachment of the muscles is shown diagrammatically in fig. 44. Electron micrographs of sections through this region are shown in Figs. 45 & 46. Figs. 45A and B show the attachment and innervation of smooth myofibrils. Processes from the myofibrils pass between the decalcified ossicles into the nerve mass. Figs. 46A and B demonstrate the same features of the attachment of striped muscle. A mass of nerves lie within the cavities of the jaw behind each apex of the triangular shape of smooth and striped muscles. Fig. 47A shows the fibrils from the muscle bundles of two sides of the triangle converging upon each other and attaching to a line of ossicles stretching out towards the mid-point of the triangle.

The processes from the fibrils that lack myofilaments (fig. 47B) penetrate the nerve mass of the jaws and abut against neurons containing a mass of small clear synaptic-type vesicles, which have a diameter of 40 mμ. These are the only recognizable contacts between the muscle fibrils and axons and

Fig. 43. Diagrammatic representation of a striped muscle fibril. Each fibril is a separate unit. Mitochondria, (m) occur in a longitudinal ridge at one radius of the fibril. A well developed T-system is present that envelopes the myofilaments as a circular tube (ts) at the level of the Z-band. The circular elements are joined by collaterals. Only the thick myofilaments are shown. (Not to scale).

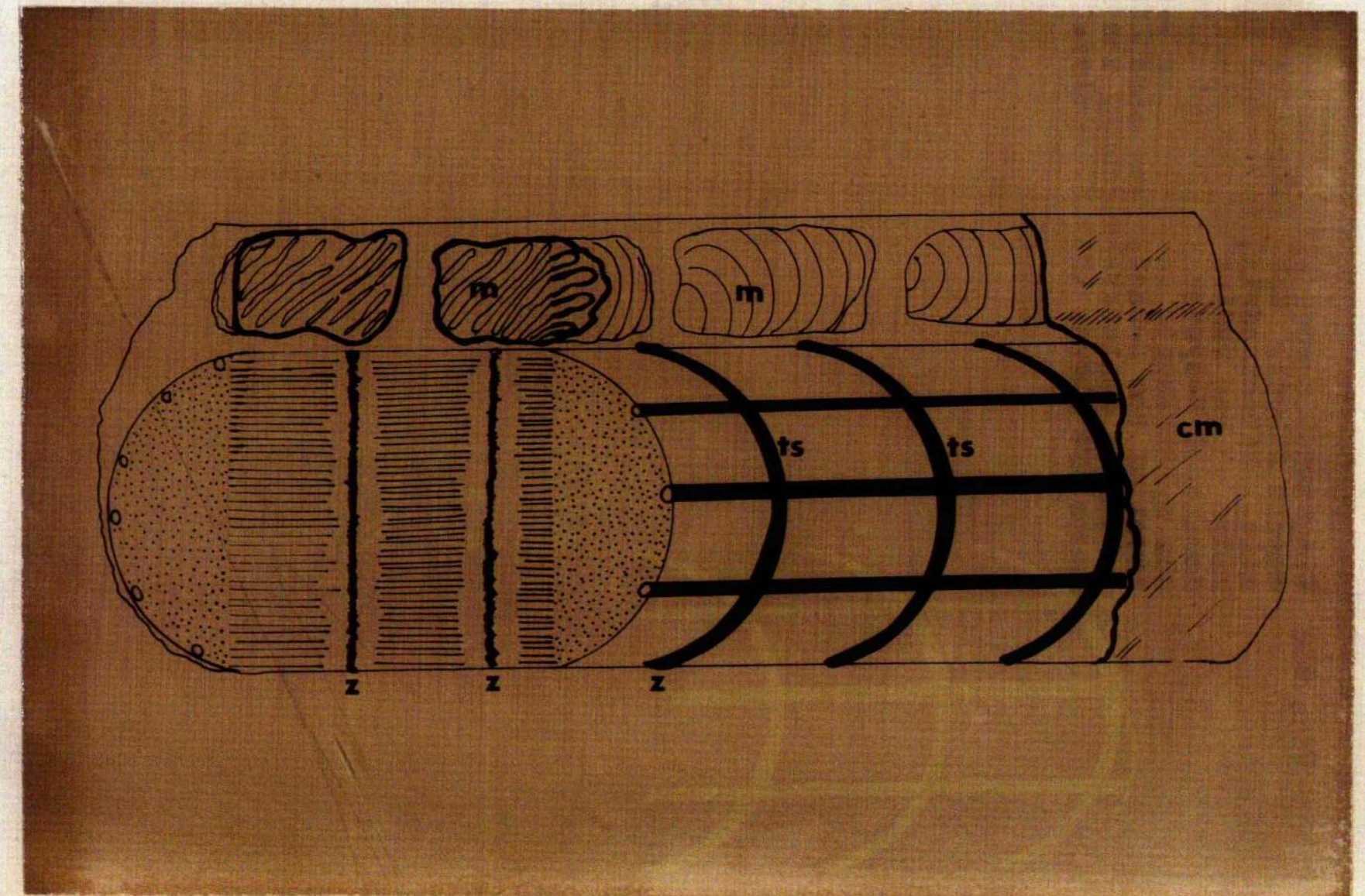

m
m
ts
ts
cm
z
z
z

Fig. 44. Diagram of the innervation of the muscles of the pedicellariae of Echinus. The closing muscles of a pedicellariae form a triangle and are innervated at the apices of the triangle. The single fibrils of both the smooth and the striped muscles (st) break up into a number of finger-like projections which attach to the ossicles and penetrates the underlying nerve mass, (nm).

st
os
CP
os
CP
os
st

Fig. 45. A: Low power electron micrograph of the nerve mass (ax) of a single jaw of a pedicellariae. The ossicles (os) surrounding the nerve mass have been decalcified. A single pore through the layer of ossicles is shown and this is penetrated by processes from the muscle fibres; the muscle (sm) is smooth (Versene treated).
B: Higher magnification of part of fig. 45A. The clear processes usually can only be traced from muscle fibrils into the nerve mass (ax) by semi-serial sections. The muscle fibrils embed in a mesh of fine collagen fibres (cn). Some axons contain dense-cored vesicles (dv). (Versene treated).

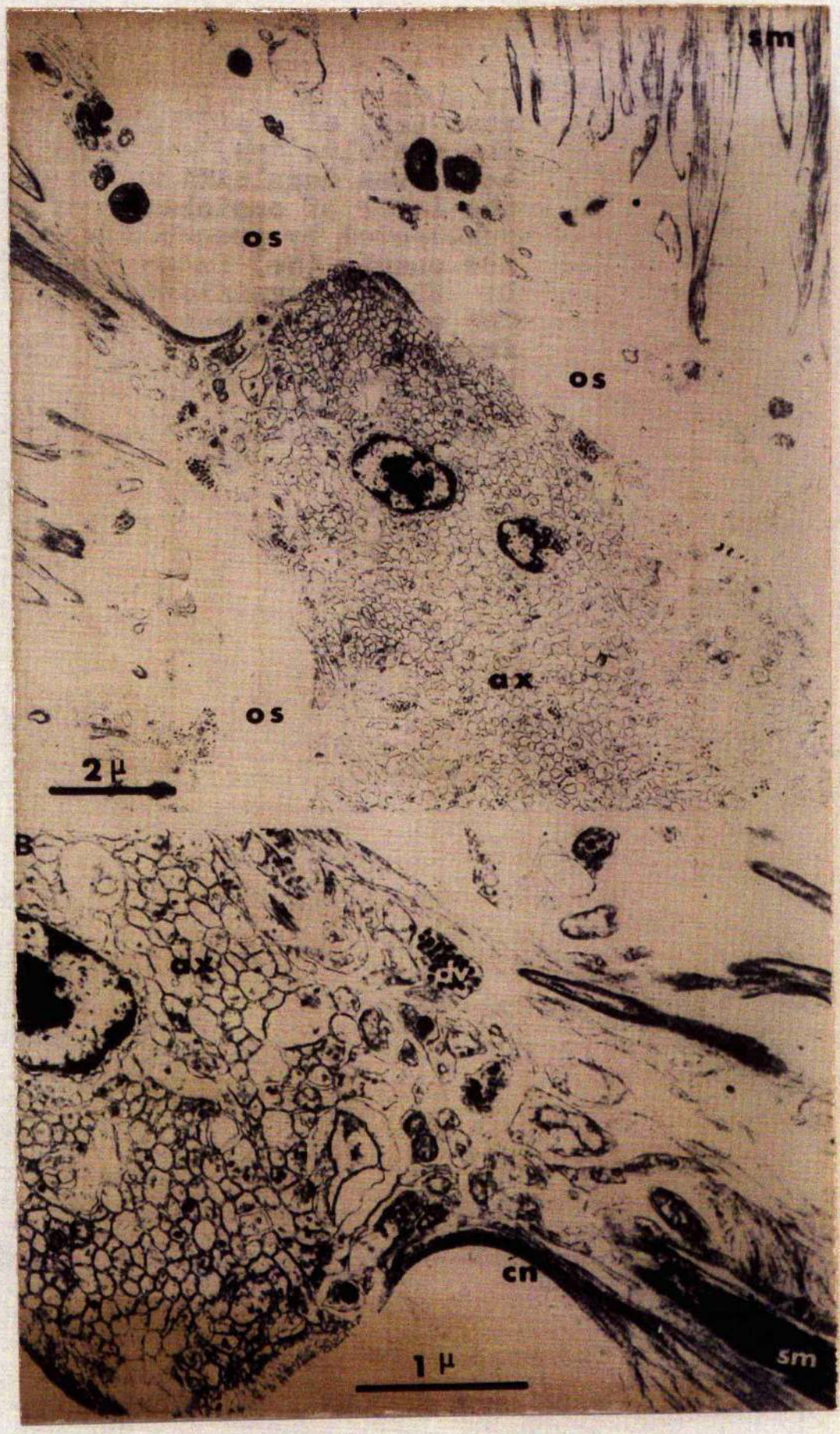
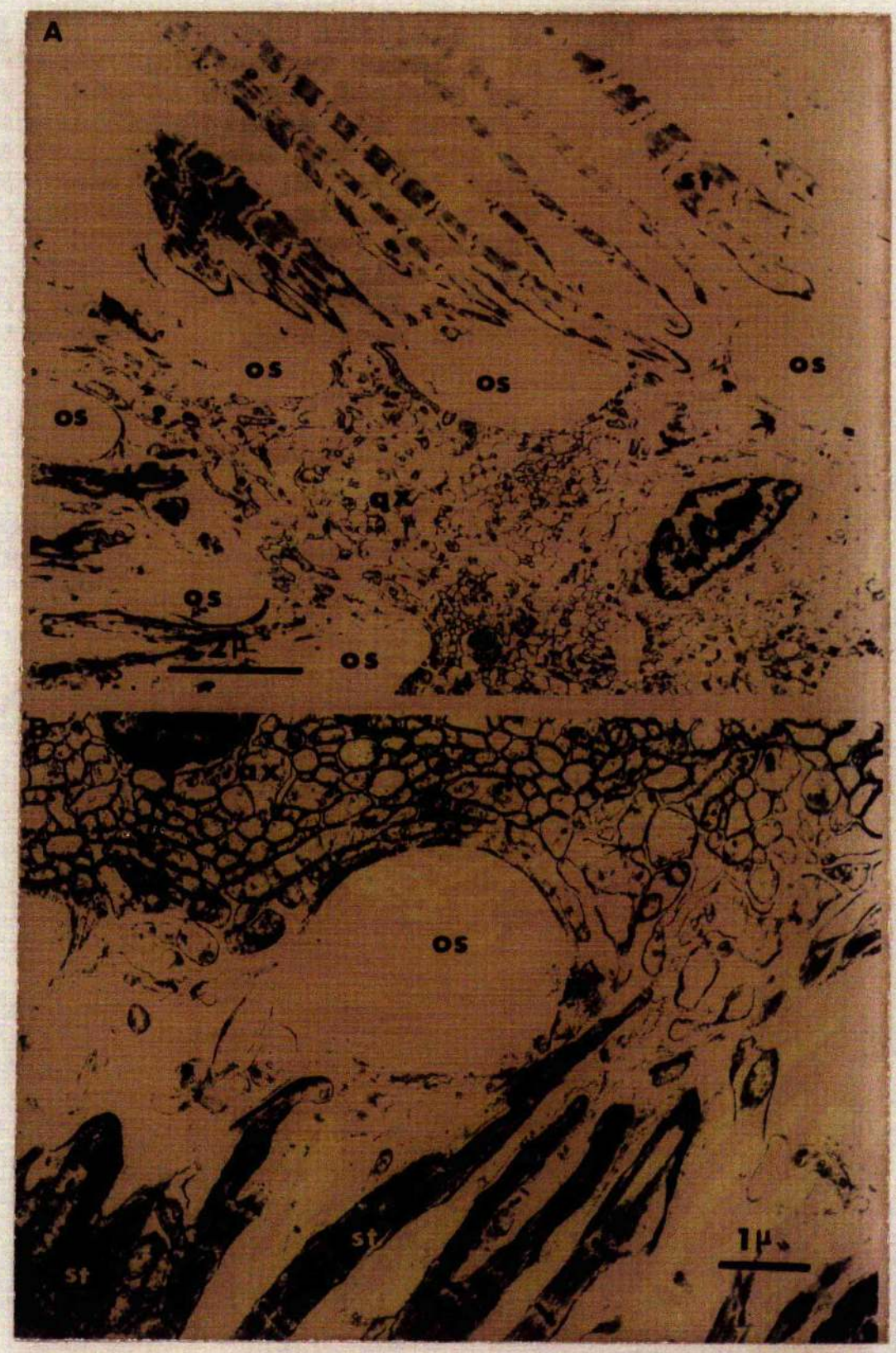

sm
os
os
ax
os
2μ
B
ax
cn
1μ
sm

Fig. 46. A: Electron micrograph of the nerve mass at a lower region than fig. 45 and thus showing the striped muscle fibrils (st). The pores in between the decalcified ossicles (os) can clearly be seen. The ossicles enclose the main nerve mass (ax). B: Micrograph at a higher magnification than fig. 46A showing pores between the ossicles (os). It should be noted that usually it is only possible to trace processes from the muscle fibrils (st) to the nerve mass(ax) using serial sections. (Versene treated).


A
OS
OS
OS
OS
OS
OS
qx
OS
B
qx
OS
st
st
1μ

hence presumably represent the synaptic site. There do not seem to be specializations of the cell membrane at the junctions The neuromuscular junctions can only be studied in versene treated material and at high resolution the cell membranes and the unit membranes of the vesicles present a granular appearance when compared to untreated material. For this reason it is not possible to describe accurately in detail the neuromuscular junctions. There is no observable difference between the innervation of the smooth muscle fibrils and that of the striped fibrils. Areas interpreted as neuromuscular junctions are shown in figs. 47C and D.

Fig. 47. A: At each apex of the triangle of muscles of a pedicellariae a ridge of ossicles (os) projec between the converging muscles (sm). Clear processes (cp) from these muscles pass up the line of ossicles to the nerve mass within the jaws. (Versene treated). B: Three clear processes (cp) from striped muscle fibrils are shown. These pass through pores between the ossicles and make synaptic contact with axons from the nerve mass. (Versene treated). C and D: Neuromuscular junctions between axons (ax) from the nerve mass and clear processes (cp) from the myofibrils. The clear processes from the muscles contain irregular sized vesicles and mitochondria. The axons contain synaptic type vesicles (sv) and occasionally dense-cored vesicles (dv). The processes can be traced back to the muscle by serial sections. (Versene treated).


A
os
os
os
sm
sf
cp
cp
cp
0·5μ
cp
0·25μ
cp
div

Types of vesicles in echinoderm nervous systems.

There are two types of clear synaptic vesicles that can be separated by their different diameters. The most common vesicle measures 400 Å in diameter and the other smaller vesicles, which are rarely found, measure 250 Å on average.

There are seemingly at least four types of dense-cored vesicles. Most commonly there are vesicles measuring 700-1000 Å and containing a core of electron dense material surrounded by a clear space inside the membrane of the vesicle. There is another vesicle of the same type but of a smaller average diameter, namely 400 Å. There are two other types of dense-cored vesicles where the electron dense material fills the entire vesicle. In all the types mentioned above the structure of the vesicle is formed by a surrounding unit membrane. The smallest of the partially-filled vesicles and both the clear vesicles appear to be involved in synaptic contacts. The large clear vesicles and the large partially-filled vesicles however are often contained in axons with no apparent synaptic contacts and there are sometimes thousands of vesicles involved, these axons filled with vesicles are some of the largest so far found in echinoderms.

In many cases the function of the vesicle containing axons is not clear though the large partially densed-cored types are often associated with neurosecretion in other phyla. There is no direct evidence for neurosecretion as yet although conventional staining techniques would indicate that it occurs

as would behavioural studies. In developing nerve cord, golgi bodies that apparently produce vesicles are visible and in some cell bodies there are large golgi complexes surrounded by vesicles. It is not certain however whether all the types of vesicles described above are separate types or whether they represent different stages in the development or utilisation of a lesser number of distinct types, nor is there any direct evidence as to what transmitters are involved in each type of vesicle though the transmission of impulses across cell junctions appears to be cholinergic.

Synaptic structure in echinoderms.

Previous to this work there were no published records of synapses within echinoderms. Careful investigation of several echinoderm preparations have shown that they occur in certain set locations. The areas where they occur generally consist of a massive interweaving of nerve fibres containing many synapses. As there are no cell bodies to the axons within these areas they may be considered as neuropile, (see Bullock and Horridge 1965, glossary). The organisation of the three neuropiles so far examined are described in later sections but as the structure of the synapses in both asteroids and echineids is apparently similar these structures will be considered under a single heading.

The identification of synapses within any nervous tissue using the electron microscope is difficult. In some phyla, containing species that can be considered advanced, (such as the decapod crustacea and the octopus and squids and also in the chordates) there are marked and characteristic specialisations of the membranes of the nerve cells at the junction, apart from the presence of vesicles. These specialisations are only shown satisfactorily in most cases using special techniques of fixation and staining. The actual structures present at such synapses depend upon type of synapses examined, the area at which they are located etc., and attempts have been made to associate these structures with known function within the nervous system of particular

transmitters and to form current hypotheses to explain the phenomenon of synaptic transmission as a whole. It was the purpose of this present study however merely to establish the identity of synapses as such to enable the location of neuropile to be made and to give coherence to a plan of the nervous anatomy at a fine structure level.

Having used a very wide variety of staining and fixation techniques it is possible to describe the presumed synaptic regions in echinoderms. Fig. 43 illustrates a number of synapses from different areas of neuropile with different staining methods. At the presumed synaptic contact the vesicles within one cell are concentrated against the cell membrane as well as partially filling the cross section of the axon. In a few axons the vesicles are lined up on either side of the synaptic contact and it seems possible that in these cases the synapses are bipolar, fig. 43. In some examples, the vesicles are of the dense cored variety and this possibly indicates that two types of synapse, involving different transmitters, are present. Fig. 48 shows that in certain synapses a clear cleft is present between the pre-synaptic membranes and the post-synaptic membranes. In other synapses the membranes appear to fuse at the synaptic contact. The last type is found with such regularity that it must be regarded as a typical condition of echinoderm synapses in fixed material examined with the electron microscope. It has not been possible to decide with certainty the relationship between

Fig. 48. Six examples of synaptic areas in echinoderms. A. Typical unipolar synapse showing cleft, slight thickening of the unit membranes and numerous synaptic type vesicles. B. Large axon containing various vesicles but one group of synaptic vesicles appears to be lining up against the cell membrane, this may be close to a synaptic area. C. Another structure considered to be synaptic, in this case there appears to be a certain amount of fusion of the pre- and post-synaptic membranes. D. Structure interpreted as a bipolar synapse. Note the faint thickening of the unit membranes and the mass of vesicles in both axons. E. Aggregation of synaptic vesicles in large axon, however there are no obvious synaptic contacts with other axons. This figure illustrates the difficulty of interpreting synaptic structure in echinoderms. Fig. F. In one instance the membranes of two adjoining axons appear tightly fused together. This could possibly represent a so-called "tight junction", however the quality of the micrograph is not of sufficientl; high standard to resolve this.


A
B
E

these synapses showing a cleft and those shewing a fusion of the membranes but it seems most likely that a synapse consists of an area where the membranes are fused surrounding an area where there is a cleft between them.

Gray (personal communication) has said that unless specialisations can be shown post-synaptically (usually using phosphotungstic acid stain) synapses cannot be positively identified. However in echinoderms all the varied methods tried to show the specialisations failed and it seems unfair to expect an investigator to show a structure seemingly not present before the identification of synapses can be confirmed. In echinoderms therefore the definition of a synapse does not include post-synaptic specialisations. Whatever the arguments may be on this subject it is clear that the structures described above are the only identifiable contacts between axons and until evidence is levelled against this hypothesis the identification must stand. The same may be said of the neurociliary synapses described by Horridge and Mackay in coelenterates, though always in the identification of this type of synapse the mere presence of a few synaptic vesicles should not be taken as indicating a synapse, but rather the lining up of them against the cell membrane. Synaptic vesicles in small numbers and occasionally in aggregations are found throughout the nervous systems (i.e. in the radial cord of Astropecten close to the layer of collagen separating the

ectoneural and hyponeural tissue and in the hyponeural ganglia of Echinus). There are no descriptions of tight junctions or other structures associated with electrical transmission nor have any been described during the present study. Bargmann and Behrens (1963) imply such structures exist in the ampullae of Asterias, as does Prosser et alia (1965) in the description of electrical activity in the gut of various echinoderms but as yet there is no structural evidence for it.

The hyponeural tissue of Echinus

General description

There are ten hyponeural ganglia associated in pairs at each radius. Figure 49 is a diagrammatic representation of the major features of the tissue. It is a complex mass of interwoven nerve fibres invested in epithelium (coelomic covering) and connective tissue. The ganglion has a small tract of fibres originating from the proximal end to the radial cord that link it with the circumoral ring; this is probably composed mainly of interneurons. On a gross scale, however, there are two main efferent tracts leading to the lantern muscles, branching at some distance from the ganglion. Sections through selected areas of this ganglion are shown in figures 50, 51 and 52.

The ganglion is separated from the nerve tracts of the circumoral ring by a block of connective tissue. This is continuous with a large mass of connective tissue that underlies the ganglion and rises up in a small hillock against the circumoral ring. The nervous tissue is further invested and separated from the connective tissue by a diffuse layer of muscle cells. In some parts of the ganglion the muscle cells and nervous tissue are intermingled.

Two morphological features of the ganglion are worthy of mention. These have been termed the hyponeural spur, a piece of tissue that projects from the main ganglion, and the hyponeural boss which constitutes an area of neuropile that

Fig. 49. Diagrammatic representation of the hyponeural ganglion seen from above (for details of the gross disposition see part I, Cobb & Laverack 1966a). The regions indicated are the hyponeural boss (hyp.b.) and spur (hyp.sp.) together with the connective tissue (c.t.) and muscle (m) inserts. Cell bodies (c.b.) lie to one side and the motor nerves to the protractor muscle (pro.n) and retractor muscle (ret.n.) originate in this area. The input channel (i.c.r.) from the circumoral ring (c.o.r.) is shown adjacent to the cell body area.

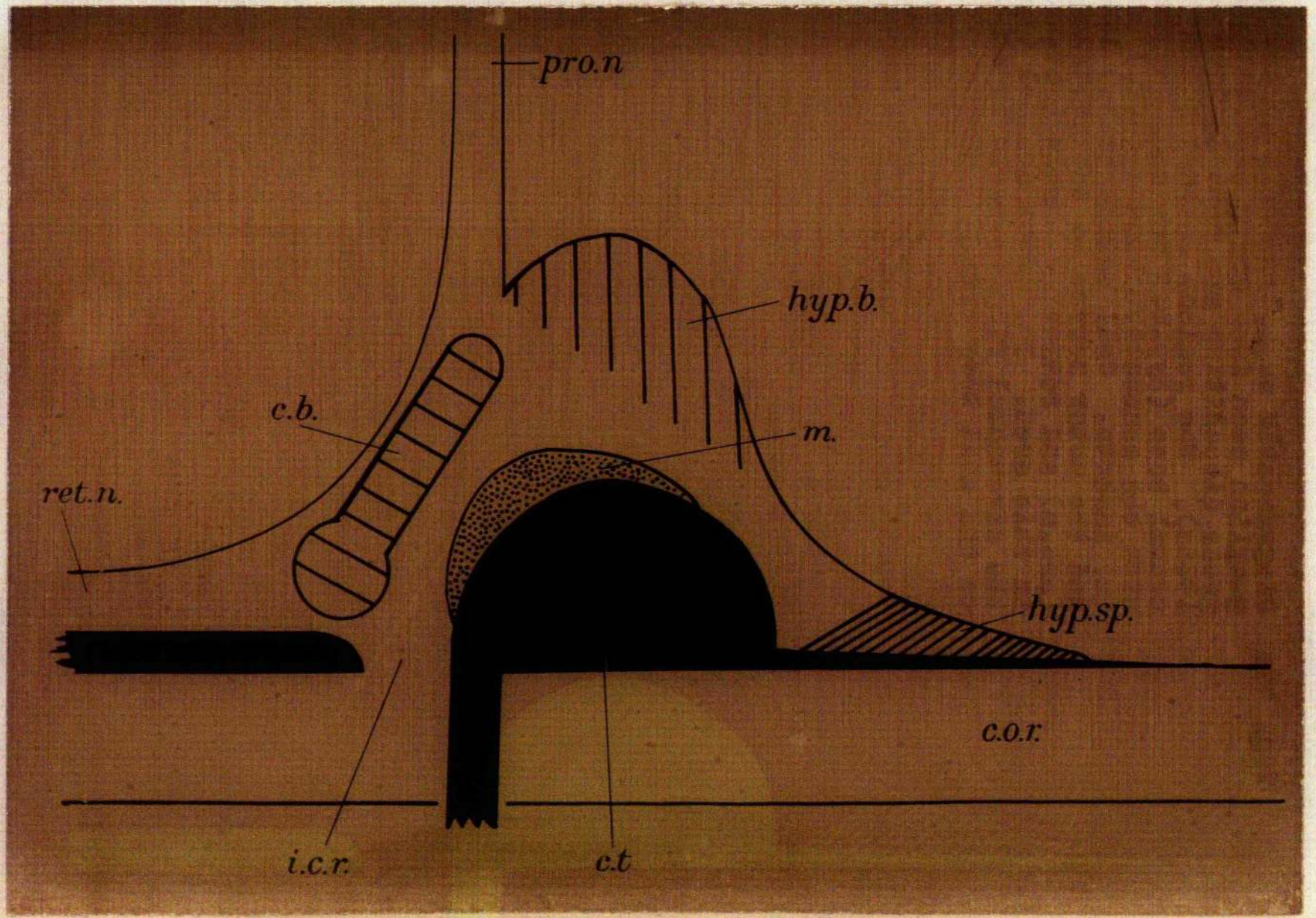

pro.n
hyp.b.
c.b.
m.
ret.n.
hyp.sp.
c.o.r.
i.c.r.
c.t

Fig. 50. Low-power electron micrograph of hyponeural ganglion. The section was taken close to the region from which the protractor muscle motor tract has its origin. Glia (g) is restricted in distribution, and there is little subdivision into bundles of fibres. The ganglion is bounded by epithelial cells (ep). Muscles (m) are found within the ganglion. The axons (a) are small, some contain mitochondria, and all contain neurotubules (OsO_4 fixation).


g
g
g
a
m
m
5μm

Fig. 51. Low power electron micrograph of hyponeural ganglion. This section is from the area close to the position of cell bodies of the retractor muscle nerve tract. Two of these cell somas (cs) are visible, and the apparent large diameters of some of the axons may indicate the axon hillock (axh) regions of yet other neurons. A tract of small fibres (sf) enters from the circumoral ring. Muscle cells (m) and epithelial boundary cells (ep) are both seen (PTA fixation).


cp
axh
cs
cs
m
sf
m
5μm

Fig. 52. Hyponeural ganglion. This section can be compared with those shown in figures 50 and 51. This figure represents an area of neuropile. The axons (ax) show many inclusions particularly numerous at arrowed positions). A chromatophore is represented by the large apparently empty cell (cp). In an area such as this up to 20 synaptic contacts as described in the text, may be found (glut/ OsO_4).


ax
cp
5μm

overlies the main bulk of the ganglion. Their significance is discussed later.

Fine structure of nerves (particularly in the hyponeural area

Connective tissue

The hyponeural area of echinoids is a complex structu It is invested in a considerable amount of connective tissue. This material forms a band beneath the ganglion, separating i from other structures, and also penetrates among the nervous tissue, breaking the structure up into subunits. The connect- ive tissue wall is about 250 µm wide and 150 µm deep where it is most extensive (figure 53A). It is composed of interwove fibres that are striated. The banding has a major repeat period of 630 to 650 Å, and closely resembles authentic collagen described in vertebrates (figure 53B).

The connective tissue surrounding the hyponeural regi is continuous with a prolongation of tissue bridging the circumoral ring and attaching to the gut connective tissue.

Muscle cells

Interposed between the connective tissue sheath and the neural contents is a layer of muscle cells (figure 54). These cells are not packed closely together. They vary in shape and size, but typically are some 8 µm long and 3 µm broad when seen in section. Many appear elliptical in shape. In fine structure parts of the cytoplasm often appear almost without content, or with occasional aggregations of mito-

Fig. 53A. Typical interwoven appearance of the investing collagenous tissue that lies around and beneath the ganglion (PTA).
B. Several collagen fibres showing typical banding. The largest fibres are about 0.2 um in diameter, and the major repeat period is 620 A (glut).

Fig. 54. Underlying the hyponeural ganglion is a layer of muscle cells (m). In some areas, as here, they are intermixed with axons (ax). The muscle cells contain myofilaments, clear cytoplasm, and the cell membranes show characteristic thickenings in places (see arrows) (OsO_4).


5A
5μm
5B
0,2μm
64
ax
m
ax
m
1μm

chondria. Many loosely packed filaments about 20 nm in diameter are contained within the cell. The nuclei are large (3 to 4 μm in diameter), and Golgi bodies have been seen.

The most striking feature of these muscle cells is the appearance of characteristic dense aggregations at certain places along the cell wall. These thickenings have been noted in material fixed in several different ways and are presumably a constant feature of the cells. The function is unknown, since their occurrence is irregular and not associated with any other obvious structural feature.

Epithelial cells

The entire hyponeural complex is covered with a layer of cubical epithelium which is but one cell thick. This indicates the coelomic cavity side of the ganglion. The epithelial layer is a loosely organized sheet, with small gaps between some cells allowing opportunities for interchange of coelomic contents with deeper lying structures (figure 55). Some desmosomal contacts have been observed between individual cells; and in PTA-fixed material there is some indication of invaginations with deep internal strands that are banded.

The outer faces of the cells are ciliated. The cilia lie in pits, the walls of which are supported by a series of 9 to 15 rod-like structures carrying folds of the cell membrane out around the base of the cilium (figure 55).

Neurons

The radial nerve cords of Echinus contain many

Fig. 55. A. Cubical epithelial cells surround the ganglion where it is exposed to the coelomic side of the lantern. The nucleus (n) is very large in relation to the volume of the cell (OsO_4).
B. The surface of the epithelial cell often carries cilia. These organelles occur in pits on the surface. The figure shows a longitudinal section of such a cilium (cil). The thickening (arrowed) in the upper wall of the pit indicates the presence of a strengthening rod that projects into each fold comprising the pit walls. (PTA).


A
n
n
1μm
B
cil
0·5μm

hundreds of axons arranged in a parallel longitudinal manner, bounded on the external surface by a layer of ganglion cells (Laverack, unpublished). The majority of these axons measure only 0.3 µm or less in diameter, with a few exceptions ranging up to 1 to 1.2 µm. The larger axons amount to approximately 3% of the total population, and contain numerous membrane limited vesicles of a type generally considered as showing the occurrence of neuro-secretion (Bern 1966).

A tract of fibres enters the hyponeural ganglion from the circumoral ring radial to the bridge of connective tissue that crosses the circumoral ring. This discrete tract, of which part is shown in figure 36B, plate 35, then divides into finer ramifications within the ganglion that become impossible to follow with any accuracy.

Within the hyponeural ganglion there is differentiation of areas. The nerve fibres are densely packed in an interwoven mesh (figure 30), save in the hyponeural spur and one or two other peripheral areas (figure 34). The average fibre size within the hyponeural complex is about 0.6 µm diameter, but this again ranges from 0.1 to 2 µm. Cell bodies of about 10 µm are also present.

There is reason to believe that both motor and sensory systems are represented within the ganglion (Cobb & Laverack 19 a), presumably interacting in the neuropile. Nerve tracts may be traced to the muscles of the lantern and within these nerves the fibres are rather larger than average diameter, being

Fig. 56. A. Axon (ax) branching occurs within the ganglion (PTA).
B. part of the tract that enters the ganglion from the circumoral ring. These are of small diameter, but one (nsf) contains a number of neurosecretory vesicles (OsO_4).

Fig. 57. This section is taken from the periphery of the ganglion in the area of the hypo-neural spur. A cell body (cs) lies at the periphery. A second nerve cell (nsv) contains numerous vesicles believed to represent neurosecretory material. Such entities are relatively common and may be up to 10 um in diameter. This section was fixed in OsO_4; in PTA-fixed material such vesicles may be clear (see figure 52 ~~xxxxx~~) 51).


57
nsv
cs
5 µm
56A
max
1 µm
56B
nsf
1 µm

between 0.8 and 1.0 µm in diameter. Within the muscle bloc nerve fibres of this size predominate and are interpreted as representing motor innervation; a minority of smaller fibre may be sensory, originating from proprioceptors that has not as yet been characterized in any other way, or interneurons.

There is considerable range of sizes in the ganglion area and it is unwise to attempt a differentiation of areas of sensory interneurons and motor fibres on the criterion of size alone. The spatial relationships of afferent and effer tracts is a function of the intermingling in the ganglion.

Glial cells interposed among the neurons are sparse in number (figure 50) as they are also in the radial cords (Laverack, unpublished).

Nerve cell bodies

Figure 1 diagrammatically shows the distribution of c bodies in the ganglion. Cell bodies occur peripherally underlying the epithelial cells bounding the coelom (figure 5 and also in particular regions among the tangle of fibres. Some scattered nuclei have been observed deep within the fibr material, but these may be the nuclei of muscle cells penetrat ing the mass.

Cell bodies are most commonly seen in lobes in which the motor tracts emanating from the ganglion have their origin The cells are alined with the efferent axons running into the motor tract (figure 51). The cells underlying the epithelium, on the other hand, show no obvious orientation.

On the basis of reconstruction from serial sections examined with the electron-microscope it appears that the nerve cells are mainly bipolar in type, with some unipolar cells among them. It has not thus far proved possible to confirm this with silver and methylene blue staining methods due to the small size of the cell bodies and their axons. Branching of the axonal processes occurs close to the cell so (figure 56). Axon branching, with the efferent fibres perhaps running to several muscle fibres from the same nerve cell, seems indicated as a major feature in the system. Many more nerve fibres have been noted in the motor tracts than have cell bodies in the ganglion.

The cell bodies are about 4 µm in diameter tapering at the axon hillock to about 2.5 µm, and giving rise to a motor axon about 0.9 µm in diameter. Branching may further diminish this size, in the efferent nerve tract. These cell bodies are thought to be mainly motor in type, although a few sensory cells may be present without distinguishing features.

Several types of inclusion may be discerned in the typical neuron. Mitochondria occur randomly in most axons bu are never very numerous except in the cell bodies. Neuro-tubules (200 Å in diameter) are also conspicuous. Their distribution shows some variation since they are more numerou in those axons nearer the centre of the ganglion (about 20 per

cross-sectional area) than in the peripheral axons (5 to 10).

Vesicles are prominent and may be classified into two types. Large membrane limited vesicles range in size from 70 to 100 nm and are known to occur throughout the nervous system in particular axons (Laverack, unpublished). In the hyponeural ganglion they often are very numerous in cells of 10 μm diameter. The majority of these cells lie peripherally just below the epithelial envelope. The vesicles show some variety between those that are completely filled with electron scattering substance, to those that show only a small core of such material and are otherwise apparently unstructured (figure 57). In tissue treated with PTA they may all have clear centres.

The second variety of vesicle is smaller, about 40 nm in diameter. They do not contain electron scattering substance and become more difficult to demonstrate after PTA fixation when the unit membrane appears less dense. They can be found singly in many axons, but typically are restricted to three specific sites. These are the neuromuscular junctions, axo-axonic synapses and in a special type of neuron.

The special neurons are found in the central region of the ganglion, and also in the hyponeural boss. They are packed with synaptic vesicles (figure 58), among which a few neurosecretory-type vesicles may be discerned. It is not known if these are first, second or higher order neurons, nor are their relationships with other axons known with any certainty.

Fig. 58. Occasional cells are packed with clear synaptic-type vesicles (syn.v). These cells are localized in distribution and are not so commonly occurring as the neurosecretory cells. A muscle cell (m) is also seen in this field (OsO_4).

Fig. 59. Transverse section through a motor nerve tract about 1 cm from the ganglion. There appear to be two distinct fibre sizes, interpreted as representing motor fibres (large, Inf) and sensory tracts (small, snf). The smaller fibres are arranged in discrete bundles. A small proportion of fibres possess neurosecretory vesicles (OsO_4).


19
sup.v.
1 µm
m
20
lnf
stf
lnf
stf
stf
1 µm

The axons of these cells are often without these massive aggregations of vesicles, and may have a diameter of only 0.7 µm compared with 3 to 4 µm at the region of maximum vesicle numbers. From the point of view of numbers and distribution of inclusions these axons are comparable with the synaptic frontal bag: amacrine cell system of Octopus optic lobe described by Gray & Young (1964).

Axon-axon synapses

Presumed synaptic regions are fairly common within the ganglion. This is the only part of the nervous system in which they have been observed so far. They are least numerous in areas where the motor tract leaves the ganglion, where the sensory input (from the radial cord) enters as a distinct tract, where there are aggregations of cell bodies, and are rarely found in the hyponeural spur. They are commonest in the hyponeural boss and in the neuropile between the two motor tracts. Comparison of figures 50 and 52, show that in some areas the axons seem to contain no ordered structures (figure 50), whilst elsewhere (figure 52) they contain many inclusions largely collections of synaptic vesicles (see figure 58). In an area such as that shown in figure 52 there might be up to 20 or more synaptic contacts.

Hyponeural boss

Lying between the hyponeural spur and the motor tract proceeding to the protractor muscles is a bulge of tissue.

This tissue has been carefully studied with relevance to physiological studies that indicate the origin of the delayed muscle contraction from this area.

It appears to be an area of considerable nerve interaction. There are relatively few cell bodies, the majority o the region being packed with axons. The size of fibres falls within the general range already described. Many presumed synaptic areas have been found in this area and there are also large vesicle-containing cells of both types. It has not yet proved possible to identify the nerve tracts that run into this region though it appears distinct from the area of derivation of motor innervation of the protractor muscles.

Motor tracts outside the hyponeural ganglion

Tracts of nerve fibres innervating the muscles of the lantern were first located morphologically by methylene blue staining (see Cobb & Laverack 1966a). These motor nerves have many branches to particular muscle blocks; for example, the branch that goes to the comminator muscle innervates the whole length of this muscle by way of small branches along the way.

Figure 59, an electromicrograph section of a tract of nerve fibres forming part of the motor innervation shortly after leaving the ganglion (about 1 cm away). There are few interposed glial cells and the tract as a whole is invested only with a thin envelope of epithelial cubical cells. There

seem to be no other supporting structures.

The axons of the motor tracts are found to fall into two distinct fibre size groups. There is variation about the mean figures, but one population has a mean diameter of 0.25 μm and the other 0.8 μm. It may be presumed, though there is no direct evidence, that the larger fibres are motor in type, the smaller ones representing units serving a different functi (sensory or inhibitory). Stimulation of this nerve is follow by only a stereotyped muscular response indicating single moto innervation, and this is taken as evidence of a non-motor function for the smaller fibres.

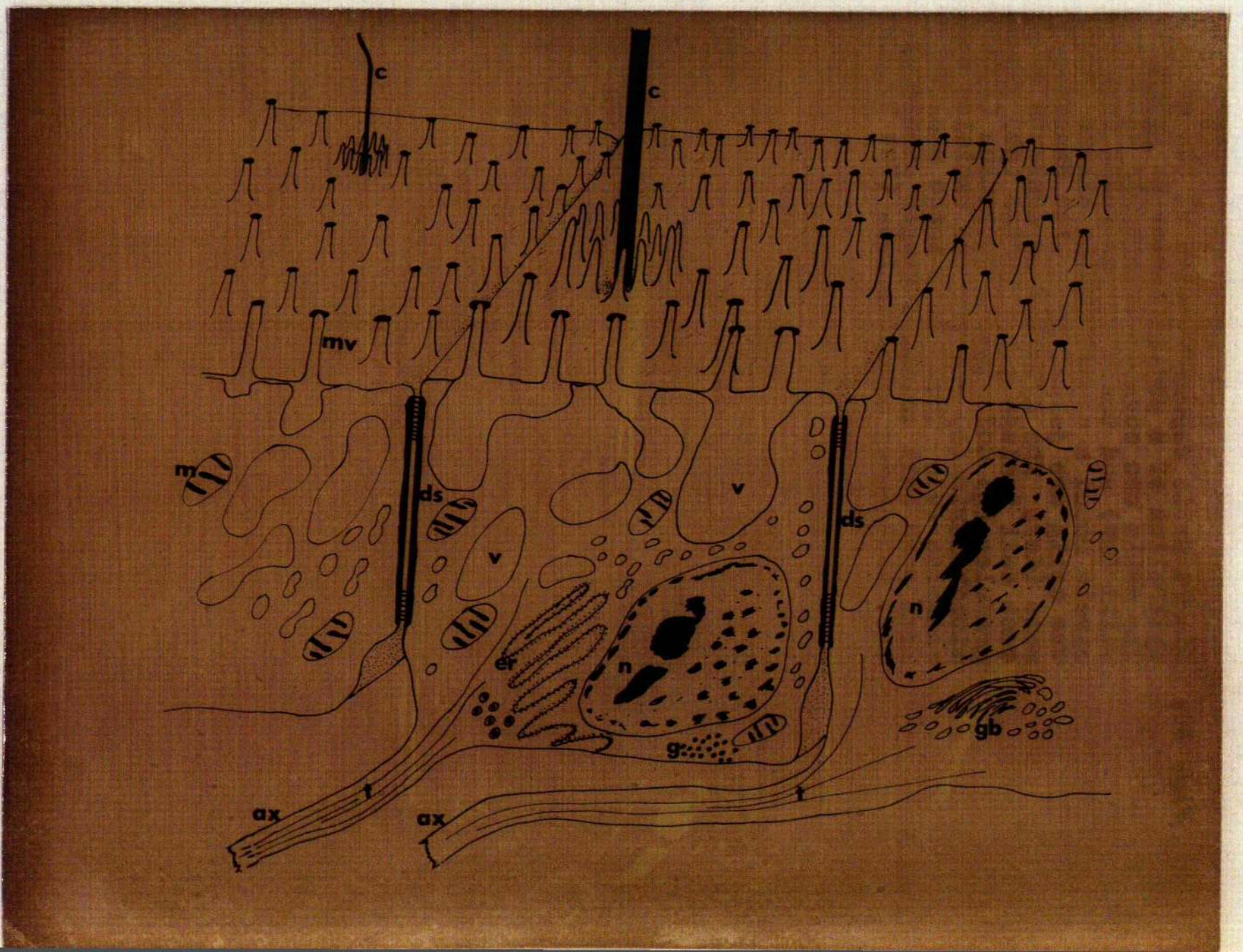
Sections cut further and further away from the hypo-neural ganglion show an over decreasing proportion of small fibres relative to the large. Amongst the muscle cells small fibres account for only 10% of the total population; close to the ganglion they account for up to 40%. This is due to recruitment and aggregation of nerve fibres from all contiguous parts of the lantern converging onto the main nerve trunk at various levels close to the ganglion. Some fibres show neuro-secretory vesicles.

General epithelium

Externally the pedicellariae are covered by a layer of epithelial cells which show certain characteristic features (fig. 60). The external surface is covered by numerous microvilli about 0.1 μ in diameter and 0.8 μ long; these microvilli terminate in small caps interconnected by fine filaments much as described by Fawcett (1966) for the microvilli of bat intestinal epithelium. The microvilli are connected to large vacuolar spaces within the cells, and these large vacuoles occur throughout the epithelial cells. Each cell contains endoplasmic reticulum, golgi apparatus and a large nucleus. There is also a variety of cytoplasmic inclusions, most commonly vesicles of a wide size range (including dense-cored vesicles), glycogen granules, microtubules and mitochondria.

Each cell bears a single cilium with a striated rootlet, the cilia are enclosed by a ring of microvilli that are slightly raised from the level of the rest of the microvilli and bear no terminal caps. The inside radius of each microvilli is thickened by an outgrowth from the basal body of the cilia. In a region extending downwards from the surface for several microns all epithelial cells are joined to each other by desmosomes. At the surface the desmosomes are septate and the same feature is present below at the termination of the desmosomal region. These structure

Fig. 60. Diagrammatic representation of the general epithelium of a pedicellariae of Echinus. The cells are covered externally with microvilli (mv) and these at one point form a circle round a single motile cilium (c). The cells are joined by a wide desmosomal zone (ds). The cytoplasmic contents of each cell include golgi bodies (gb) glycogen granules (g) endoplasmic reticulum (er) mitochondria (m) and various vesicles and vacuoles (v). There is a large nucleus (n). From each cell arises a single axonic process (ax) containing neurotubules. (Not to scale).


c
c
mv
m
ds
v
v
ds
n
ef
n
gb
g
ax
ax

are universal between all epithelial cells so far examined in echinoderms and their significance has been discussed by Eakin and Westfall (1964) and Cobb (1967a) in asteroids.

Figure 61 shows the junctions of four epithelial cells and the desmosomes separating them. The septate desmosomes are illustrated in Fig. 62 which shows a longitudinal section of an epithelial cell.

Sensory hillock

The sensory hillock (fig. 63) is formed by a collection of about 2,000 epithelial cells compressed into a circular area about 30-40 microns in diameter. At the surface of each compressed epithelial cell is a single cilium surrounded by a ring of microvilli. Below this the cells are joined together over their entire lateral faces by desmosomes to a depth below the surface of about 4 μ (fig. 64). At this level the cells average less than 1 μ in diameter, and this diameter is constant until the cells expand to contain a nucleus about 3 μ across. Below the desmosomes the cells show no specializations of the membranes and the cellular contents are similar to the general epithelial cells. At the distal side of the nucleus from the surface, the cell narrows to form a single axonic process that extends into the main tracts of nerves that run towards the area of neuropile at the base of the jaws. The rootlet of the cilia runs from basal body past the nucleus and ends

Fig. 61. Transverse section through four epithelial cells showing the desmosomes (ds) joining them. A single basal body (bb) is present in one cell.

Fig. 62. Longitudinal section through a general epithelial cell showing microvilli (mv) at the surface and various cellular inclusions. Two basal bodies (bb) are present and a region of septate desmosomes (ds) occurs at the junction with the next cell.

ds
ds
ds
1μ
inv
bb
1μ
ds

Fig. 63. Diagram of a single jaw of a globiferous pedicellariae of Echinus showing the sensory hillock (sh). The general epithelial cells (ep), the specialised epithelial cells of the sensory hillock and their axons (ax) are shown. (Not to scale).

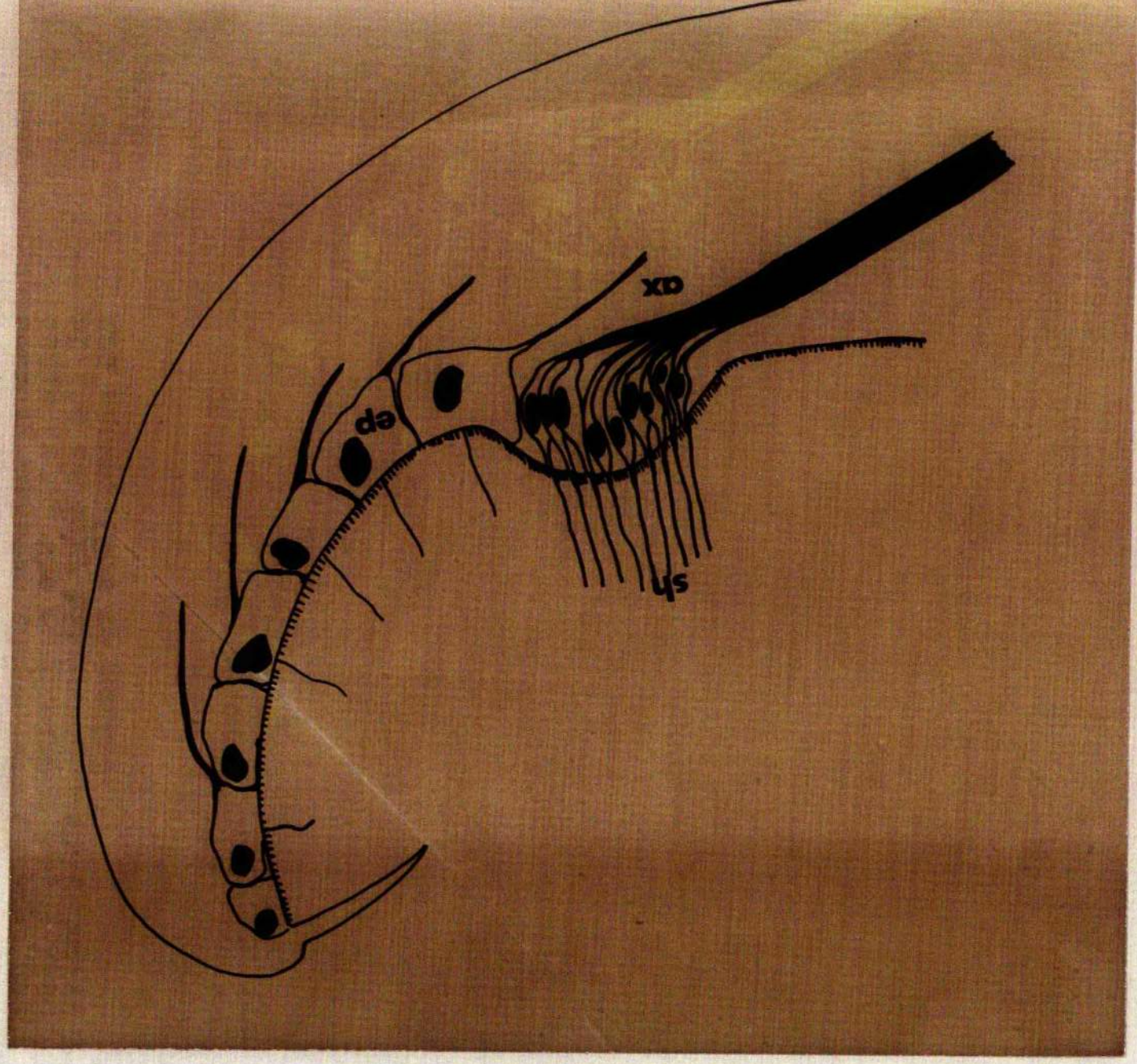
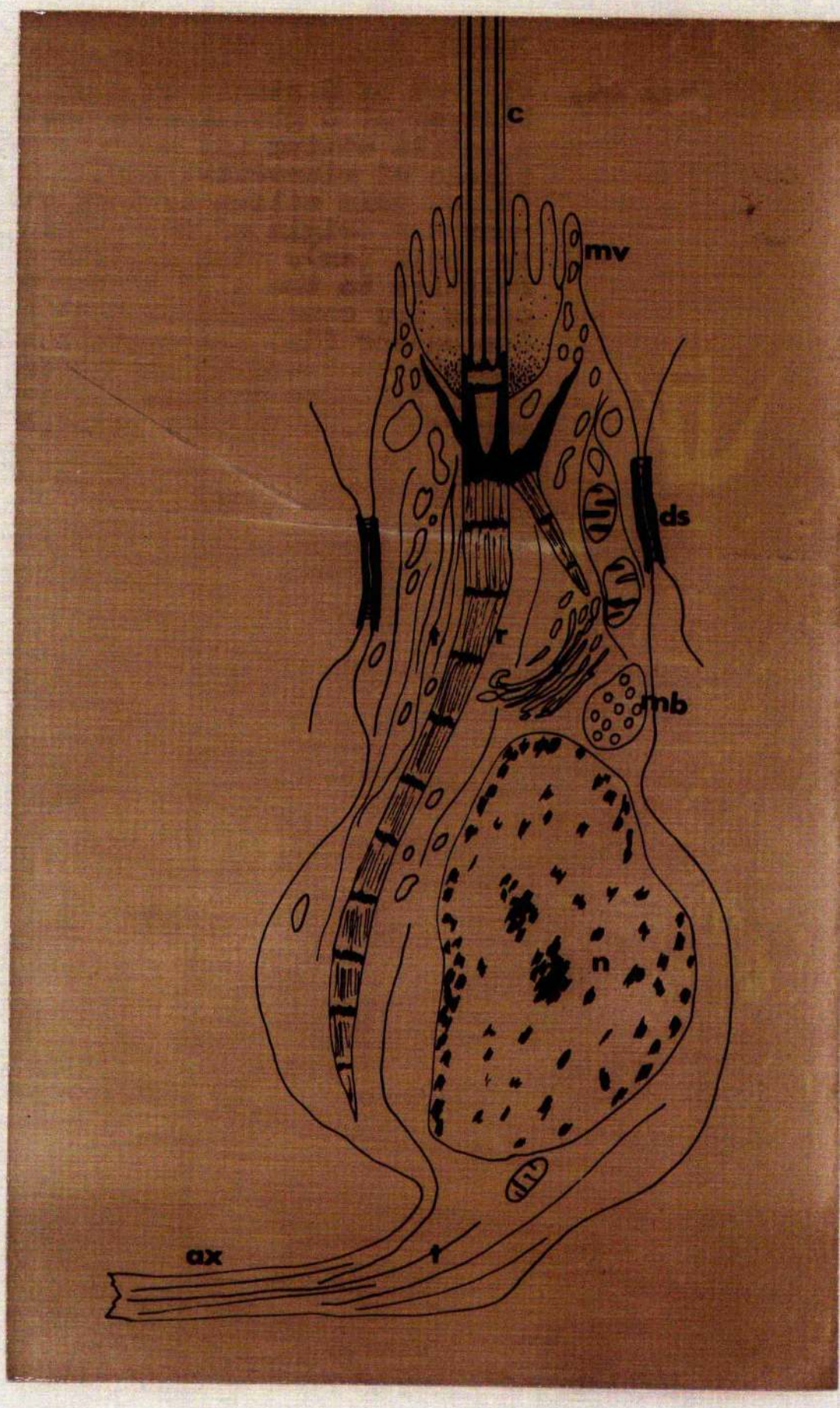

XD
dp
sh

Fig. 64. Diagram of a single cell of the sensory hillock of a globiferous pedicellariae. A motile cilium (c) emerges through a circle of microvilli (mv). The rootlet (r) of this cilium is long and terminates near the origin of the axonic process of the cell (ax). The nucleus (n) is large relative to the size of the cell. The cells are connected to each other by desmosomes (ds) and contain similar cytoplasmic contents to the general epithelial cells but also include multivesicular bodies (mb) and microtubules (t). (Not to scale).

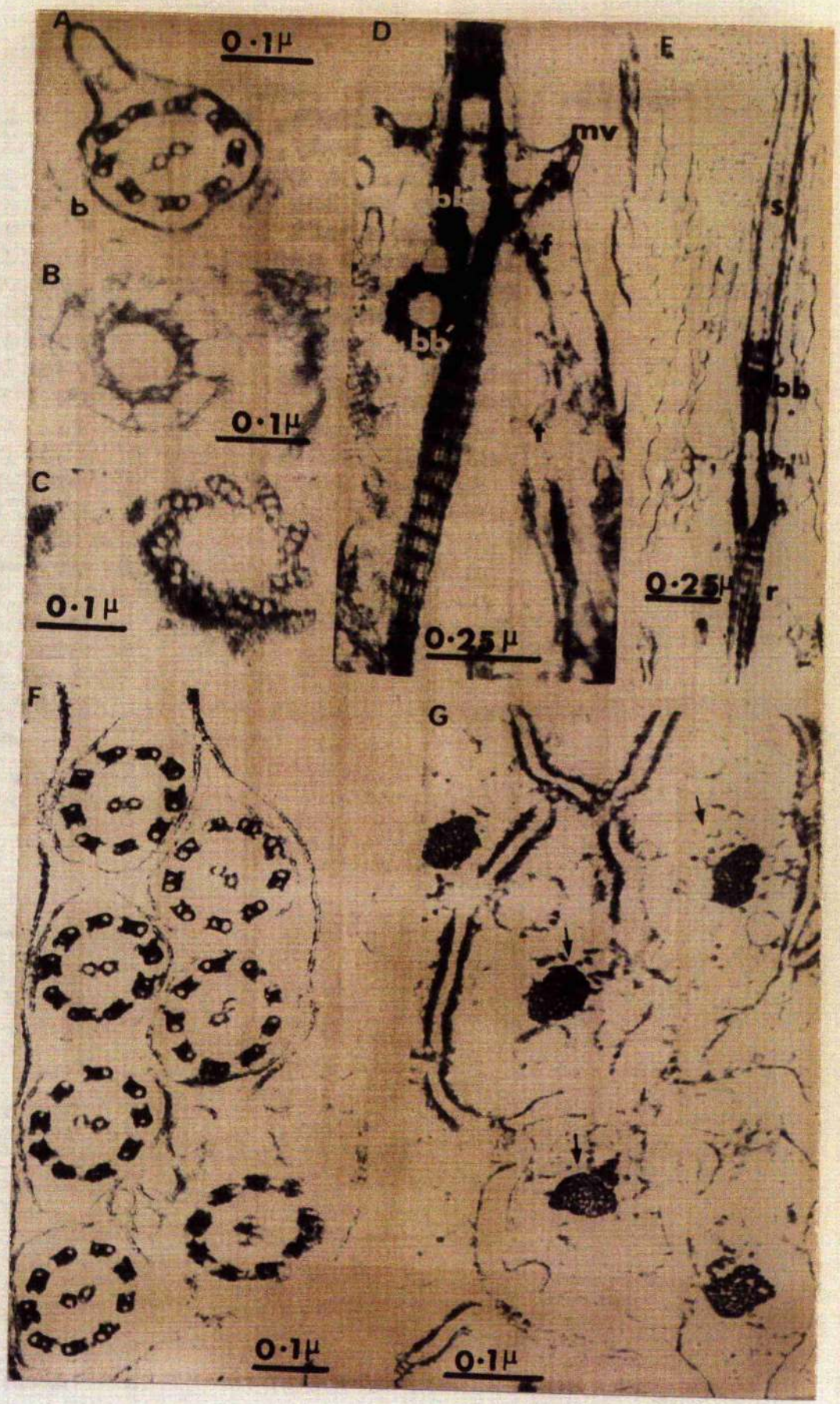

c
mv
ds
r
mb
n
ax

close to the origin of the axonic process.

Structure of the cilia

The cilia (fig. 65) of both the general epithelial cells and the sensory hillocks are very similar to those described in other material, particularly to the gill cilia of lamellibranch molluscs (Gibbons, 1961). There are nine outer doublets of fibres and two central ones joined by a series of radial links that pass through nine secondary fibres. One sub-fibre of the outer doublets bears small arms and one pair of doublets (numbers 5 and 6 using the nomenclature of Afzelius (1959)) are joined together by a bridge. The basal body consists of triplets of fibres and the transitional segment between the basal body and the main part of the cilia is similar to that described in the gill cilia of Anodonta by Gibbons (1961). A striated rootlet, occasionally divided into two parts, extends from each basal body into the cell. The striations on the rootlets show a major period of repeat of 625 Å. The rootlets are composed of a series of fine tubules running longitudinally. A basal foot is present in the region of the basal body and from here there are also connexions with the thickenings of the microvilli surrounding the cilium. In some cells, a second basal body is associated with that of the main ciliary shaft. This structure is not present in every cell and in some it is replaced by a granular body.

Fig. 65. Transverse sections of a cilium, A: main ciliary shaft, b: transitional region, c: basal body, D: Longitudinal section of a cilia. A second basal body (bb') at right angles to the basal body (bb) of the main ciliary shaft is shown. A basal foot (f) is present associated with another structure rising to the origin of one of the microvilli. Microtubules (t) surround the basal bodies and the basal foot. E: shows the ciliary shaft (s) basal body (bb) and the striated rootlet (r), F: Transverse section of cilia above the sensory hillock. Both the clockwise and the anticlockwise enantiomorphs are shown, using as a guide the direction of the arms of the outer sub-fibrils; it is not clear whether this is due to the cilia running in both directions in the plane of cut. G: Transverse section through cells of the sensory hillock below the region of the basal body showing the beginning of the desmosomes between the cells. Note the microtubules associated with the tubules forming the rootlets, (arrowed).


A
0·1μ
b
B
0·1μ
C
0·1μ
D
mv
bb'
0·25μ
E
s
bb
0·25μ
r
F
0·1μ
G
0·1μ

Second basal bodies have been observed in other sensory cells, e.g. in the lamprey, amphioxus and the tree frog (Eakin 1963).

Microtubules diverge from the basal body and the foot. In transverse section the tubules are in close association with the tubules of the rootlets (fig. 65C) and longitudinal sections through the cilia (fig. 65D & E) suggest that the microtubules may originate from the same structure as the tubules of the rootlets. In the sensory hillock of the globiferous pedicellariae the microtubules running from the basal body appear to be continuous with the neurotubules of the sensory axons. Fig. 66 shows transverse sections through different regions of the sensory cells.

Fig. 66A. Transverse section through the cells of the sensory hillock below the desmosomal region. Most of the cells contain a single ciliary rootlet (r) microtubules and various vesicles and mitochondria.

Fig. 66B. Transverse section through cells of the sensory hillock at a deeper level than fig. 66A showing a nucleus. Many microtubules are present in all the cells. The rootlet (r) is still present in the cell containing the nucleus (n).

Fig. 66C. Transverse section of the axons passing from the sensory hillock to the nerve mass at the base of the jaws. The axons contain many neurotubules and a few mitochondria (m); there are very few vesicles.


A
G
G
1μ
B
n
1μ
C
dx
px
1μ

The Radial nerve of Astropecten

The nervous system of the radial nerve cord of Astropecten is described in detail by Smith (1950) from methylene blue studies. The radial nerve cord consists basically of five layers, Fig. 67A. The oral layer consists of a mixture of sensory cells epithelial cells, (though these may be identical to the former), supporting cells (Fig. 68) and various secretory and pigment cells. Below this region are the main axons of the ectoneural tissue, the vast majority of these axons run parallel to the long axis of the radial nerve cord. Situated aboral to the ectoneural nerve and separated from it by a very thin layer of collagen is the hyponeural nerve tissue also known as Langes nerve (Fig. 69). Finally covering the hyponeural tissue aborally is a very thin covering of epithelial cells that form the wall of the radial perihaemal sinus (Fig. 70).

The epithelial cells.

The radial cord is covered orally by a microvilleous surface (Fig. 71). In the centre line of the radial cord and laterally between the podia irregularly placed cilia protrude between the microvilli. These microvilleous epithelial cells are similar to those described in an earlier section. In some cases the epithelial cells contain a fibrous structure that is enclosed in a projection of the epithelial cell that passes right through the ectoneural tissue and attaches to the

Fig. 67. Low power of radial cord of Astropecten. In the ectoneural tissue (E) large vesicle-containing axons are present against the collagen (C). The glial cells supporting the ectoneural tissue are characteristic. In the hyponeural tissue (H) there are muscle cells (M) nerve cell bodies (CB) and axons (AX).

H
E
U

Fig. 68. Epithelial cell containing glial-like fibres that pass down through the ectoneural nerve tissue and attach to the collagen layer. (see Fig. 72).


1μ
Glial
E

Fig. 69. Hyponeural tissue separated from the ectoneural tissue by a layer of collagen.


0·5μ

Fig. 70. Mixture of hyponeural nerve cell bodies and epithelial cells lining the perihaemal sinus.

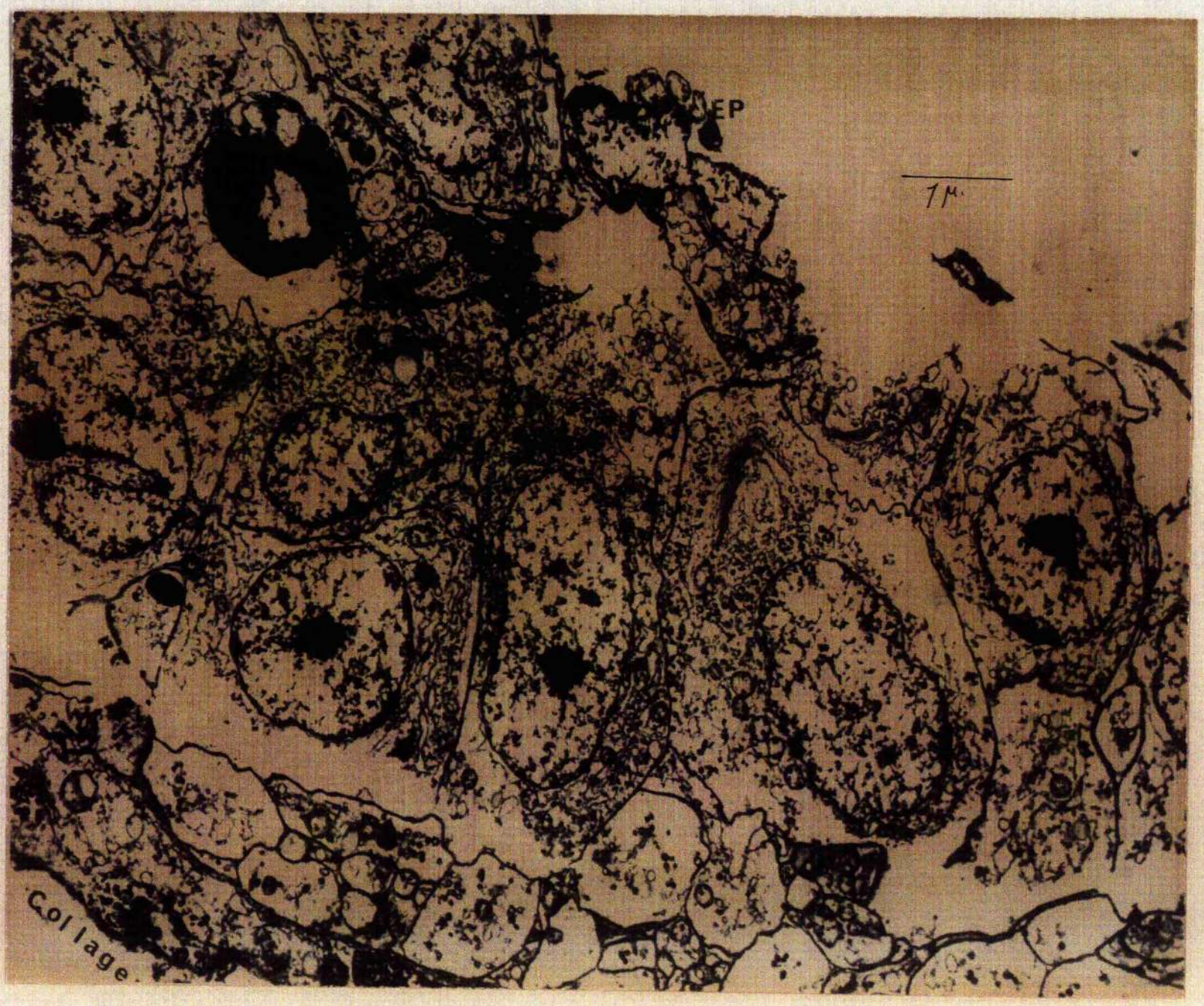

EP
1μ.
Collage.

Fig. 71. Epithelial cell covering the oral surface of the ectoneural nerve tissue. A layer of tonofilaments (T) underlies the microvilli (MV). A septate desmosome between two cells is shown (D).

Fig. 72. The border between the ectoneural tissue and the hyponeural showing the attachment of a glial fibre (G).


MV
7μ
G
E
5μ
H

layer of collagen separating the hyponeural tissue from the ectoneural tissue. These structures are shown in Figs. 68 and 72; they appear to be similar to glial cells. Their chief function would indeed seem to be that of supporting the nerve fibres of the ectoneural tissue, and giving the whole cord structural ridgidity.

The ectoneural tissue.

The nerve fibres of the ectoneural tissue are arranged such that they run in the long axis of the radial cord. The number of fibres within the ectoneural tissue depends on the overall size of the cord but it may be as many as one hundred thousand fibres, within a single cross section. Most of the nerve fibres average about 0.75 microns in diameter and are packed closely together with no non-nervous sheathing in between them. These axons are almost empty of any cytoplasmic inclusions, though there are a few scattered mitochondria and vesicles. Certain axons however contain a mass of neurotubules, Fig. 73, the distribution of these last axons is apparently at random throughout the cord and they are not associated with any particular collection of neurones within the cord.

Some of the epithelial cells apparently send axonic processes into the ectoneural tissue and therefore represent the sensory input into the cord from the exposed oral surface. These epithelial cells are very similar to those described in a later section as covering the pedicellariae of

Fig. 73. Only a very few cells in the ectoneural nerve tissue contain a concentration of neurotubules (NT). The reason for this is not clear. In the hyponeural tissue (fig. 80) and the sensory axons (fig. 66) there are a large number of neurotubules.

Fig. 74. There are large vesicle filled axons at the level of the collagen (C) separating the hyponeural tissue from the ectoneural tissue. The function of these axons is obscure. (Radial cord of Astropecten).

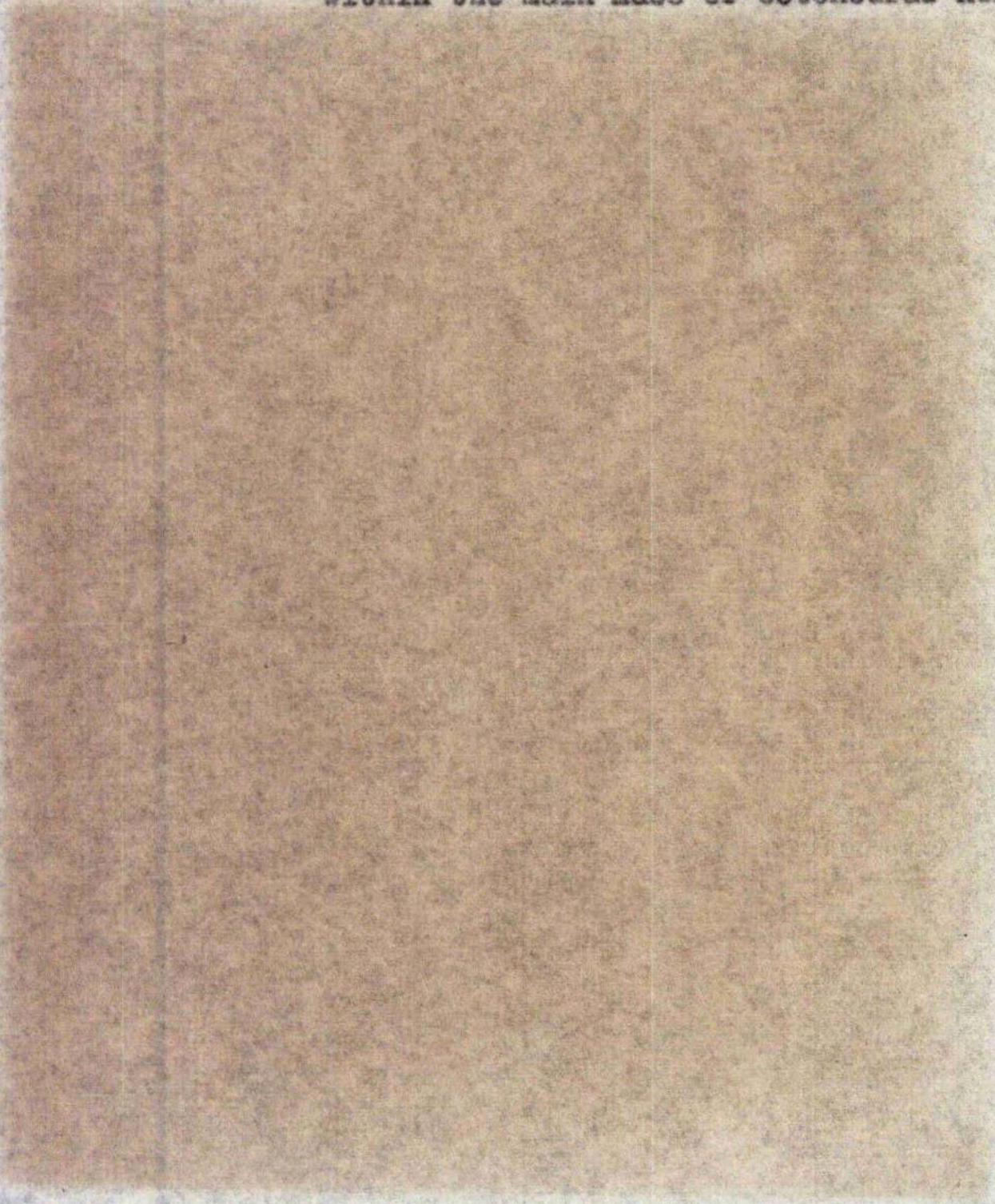

NT
1μ
C
C
1μ

Echinus. It is not clear whether all epithelial cells in the radial cord function as sensory cells or only a few of them.

The most interesting feature of the ectoneural tissue and equally inexplicable is the presence against the collagen separating the two layers of nervous tissue of a single layer of axons all crammed full of synaptic type vesicles. Several such cells are shown in figs. 74, 75. The axons containing the vesicles have a much larger diameter than the other cells of the ectoneural tissue. It is possible to trace a part of these axons containing the vesicles back into the main mass of the axons of the ectoneural tissue, fig. 76. The main mass of the vesicles appears to line up against the basal lamina of the collagen separating the ectoneural and the hyponeural tissue. At no point is there any direct connection between the ectoneural tissue and the hyponeural tissue, and no question therefore of synapses across the connective tissue. There are apparently no synapses between the vesicle filled axons, as there is no fusion of the membranes and no areas where the vesicles line up against the unit membrane. Nor are there any synapses between these vesicle filled axons and the main mass of nerves within the ectoneural tissue. In developing radial nerve from regenerating arms, the axons have considerably fewer vesicles within them, and some show golgi

Fig. 75. A layer of collagen separates the axons packed with synaptic vesicles from the axons of the hyponeural tissue.

Fig. 76. The vesicle-containing axons (sv) can be traced back into the main mass of the nerve cord. There are no obvious synaptic contacts either in the bulbed part of the axon or within the main mass of ectoneural nerves.




SV
1μ

bodies included apparently producing the vesicles (fig. 77).

Separating the ectoneural tissue from the aboral hyponeural tissue is a layer of collagen. This layer is non-cellular and varies in thickness from less than 1/10 micron to several micra. Pentreath, unpublished has shown this layer of collagen in Asterina, another Asteroid to be much thicker. In contradiction to earlier authors using light microscope methods (see Nichols 1966) there are no connections through the collagen layer and any contacts between the hyponeural tissue and the ectoneural tissue must take place outside the cord, and there is some evidence that this is so from studies of the appropriate regions.

The axons of the hyponeural tissue arise from unipolar cell bodies (though a few may be bipolar Fig. 78). These cell bodies lie to the centre of the hyponeural layer of the cord. Some of these cell bodies give rise to a single cilia that protrudes into the perihaemal sinus on the opposite side of the nucleus from the axon hillock Fig. 79, these cilia differ from all others found in echinoderms so far, in that they possess no ring of microvilli surrounding the cilia. The axons of the hyponeural tissue being about 1.2 microns are larger in diameter than the ectoneural axons they also differ in having no vesicles of any type and all containing large numbers of neurotubules Fig. 80. At the sides of the radial cord the hyponeural tissue is composed entirely of axons with no cell

Fig. 77. Some axons in Astropecten contains golgi bodies that appear to be involved with the dense-cored vesicles, it is not clear whether the vesicles are produced by such golgi bodies. Many of the epithelial cells also contain masses of vesicles and numbers of golgi bodies.


7μ

Fig. 78. The nerve cell bodies of the hyponeural axons are contained within the radial cord. The cell bodies appear to be unipolar and axons can be seen arising from the cell bodies (AH).

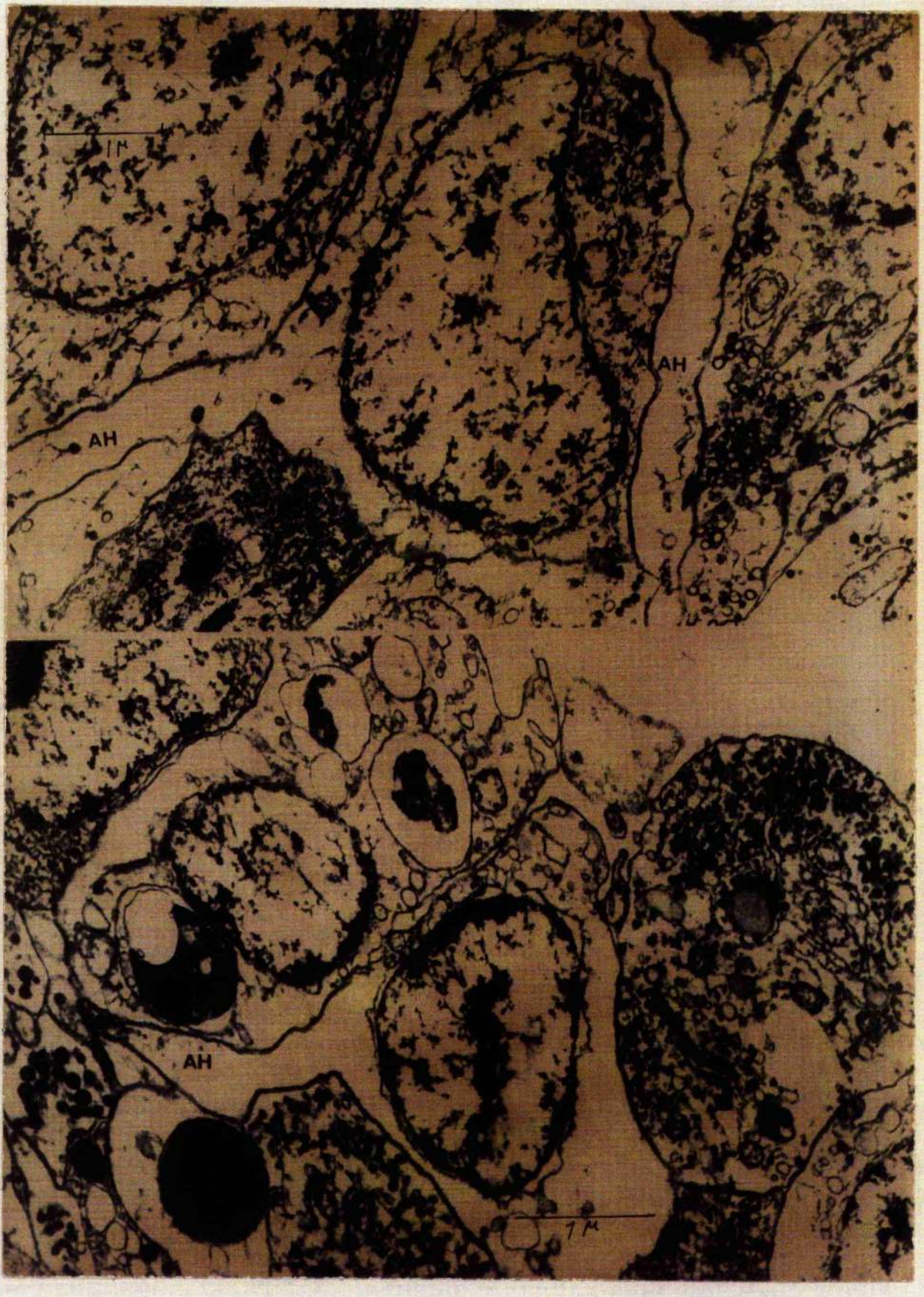

1μ
AH
AH
AH
1μ

Fig. 79. Some cells of the hyponeural nerves give rise to cilia that project into the perihaemal sinus. These cilia are unusual in that there are no microvilli associated with them. There are two basal bodies, (BB).

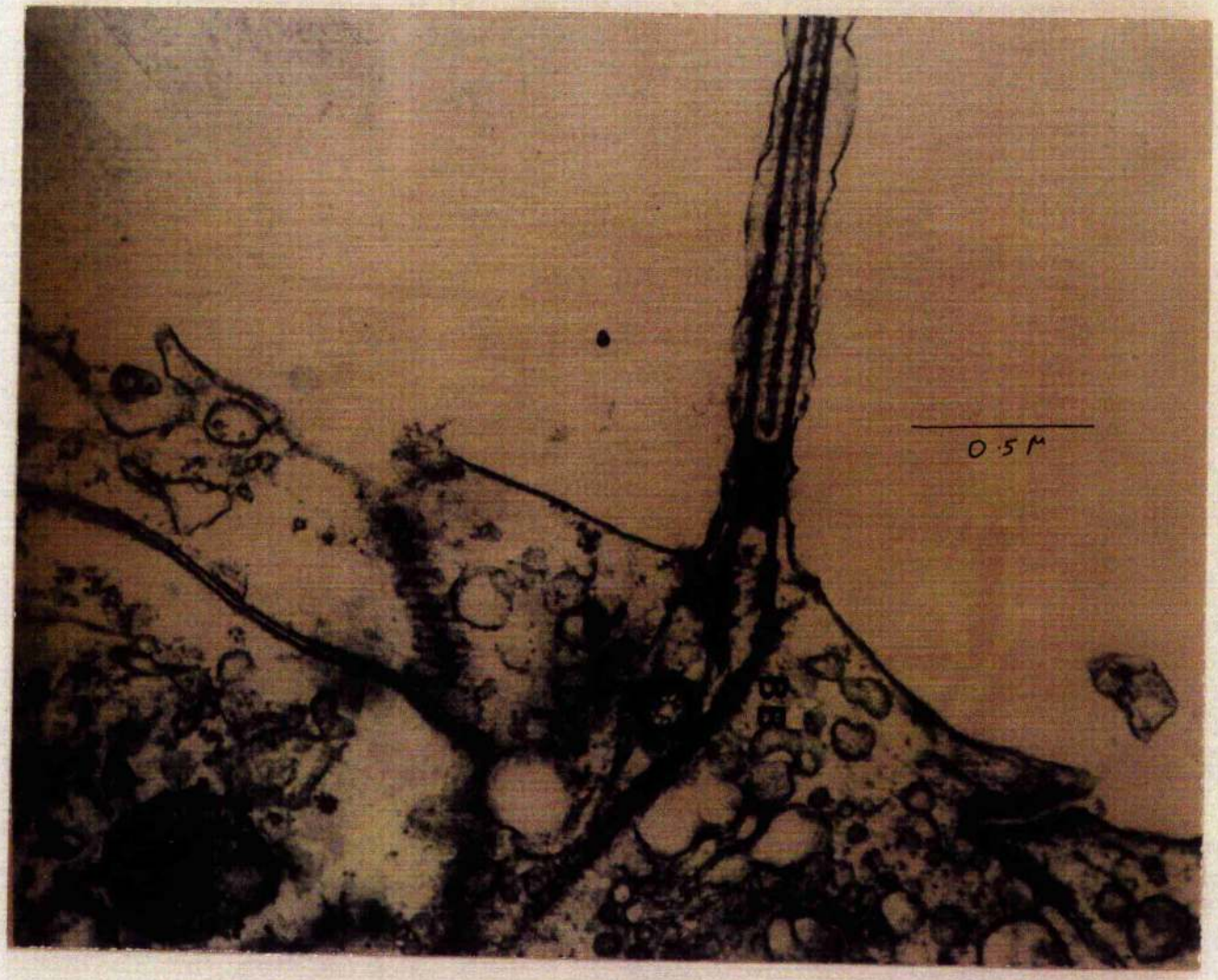
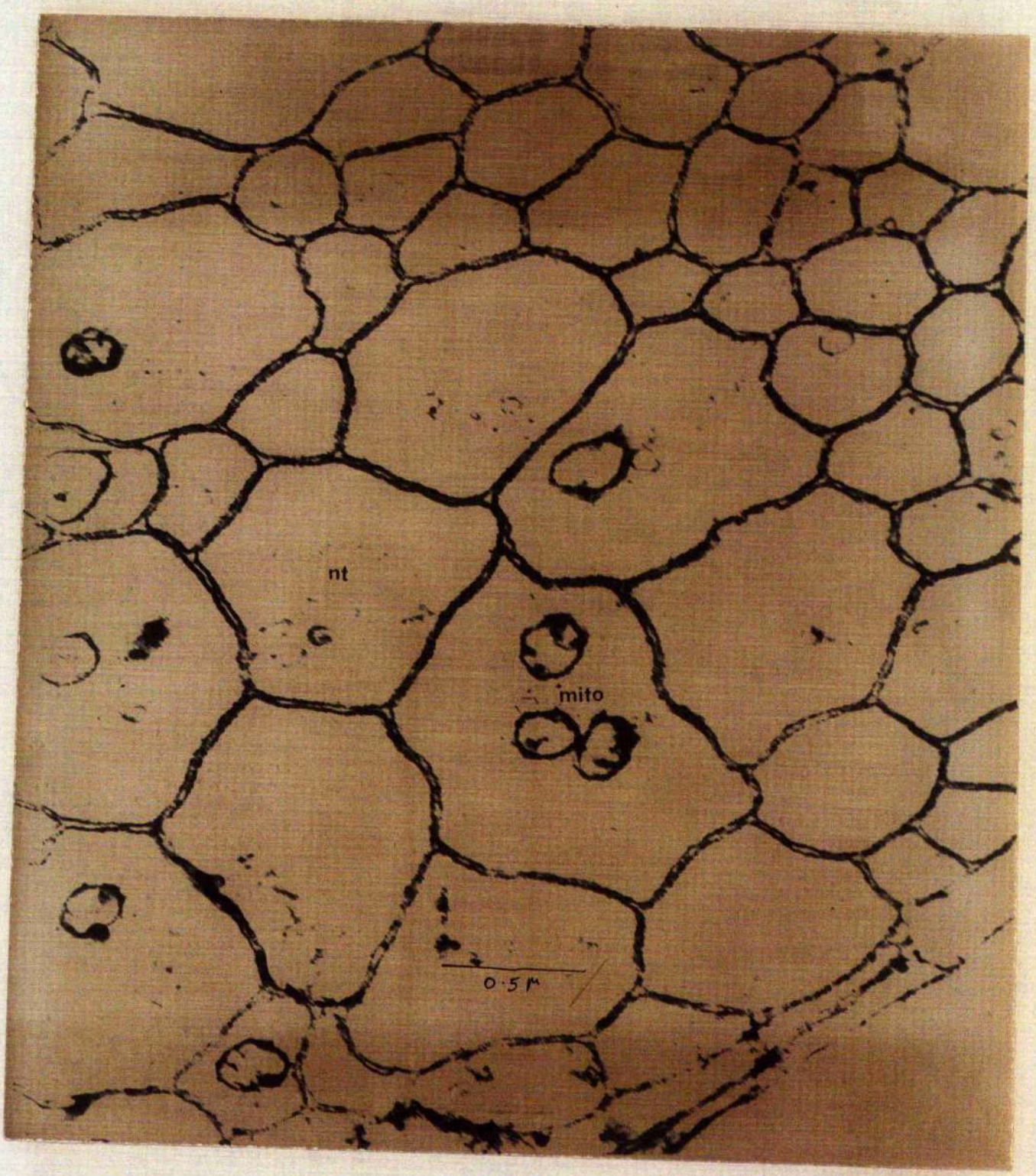

0·5 M

Fig. 80. The axons of the hyponeural tissue are of larger average diameter to the ectoneural axons and contain many neurotubules (nt), and small mitochondria (mits.)


nt
G
mito
0·5 µ

bodies. There are a few epithelial cells covering the hyponeural tissue and lining the perihaemal sinus. In between the hyponeural nerve cell bodies are numbers of muscle cells containing the same filamentous structure as the muscle cells of the seams of the ampullae.

Neuropile within the nerve cord.

Within the nerve cord of Astropecten as with other echinoid nervous systems there are numbers of axons containing dense cored vesicles with a diameter of approximately 800 Å. These axons containing vesicles are not localised to particular areas but occur generally throughout the cord. Axons containing concentrations of synaptic type vesicles however occur in definable areas. As mentioned above there are numbers adjacent to the collagen dividing the two layers of nervous tissue but these do not appear to be associated with synaptic contacts. Fig. 81 illustrates the main areas of the cord that can be considered as neuropile due to the presence of large numbers of synaptic contacts within these areas. The types of synapse found within the neuropile is discussed in a different section.

One question as yet unresolved is the distribution of cell bodies within the cord. Those of the hyponeural tissue are obvious and easily located. Those of the ectoneural tissue pose a much more difficult problem. Within the oral epithelium of the cord there is a number of cells giving off axonic

Fig. 87. Laterally and between the podia in the ectoneural tissue there is a mass of neuropile where many axon-axon synapses are found (see fig. 48). These areas are considered to be the controlling ganglia of the tubefeet.


1μ

processes into the main mass of axons lying aborally. However the number of these cells is considerably less than the total number of axons present and it seems likely that they represent the sensory input to the cord from the epithelium. There are no other concentrations of nerve cells bodies within the cord and presumably the cell bodies of the main mass of axons are located peripherally. This question should be answered satisfactorily using surgery and degeneration techniques. Early experiments however have shown that the structural changes occurring are complicated and varied; carefully controlled experiments will be required to settle this problem.

DISCUSSION

Physiological Results

Boltt & Ewer (1963) have described the physiology of the lantern muscles of another echinoid, Parechinus angulosus, in which refractoriness towards electrical stimulation of the nervous system was a noteworthy aspect of the investigation. Stimulation by light, however, was a potent influence when directed on to the muscles. In the present experiments electrical stimulation was effective both directly on the muscle, and indirectly via the nervous system.

Apart from a few inconclusive experiments all the physiological results described in an earlier section were made on the retractor muscle/hyponeural tissue preparation of the lantern or Echinus.

It is essential, however, that electrical stimulation be applied at an appropriate point. When the radial nerve cords are affected at some distance from the lantern there is no consistent observable response. Mechanical stimulation of the radial nerve cords, however, can be effective at long distances in some instances. Controllable electrical methods only become effective when applied to the hyponeural tissue which is the probable origin of the motor fibres to the musculature.

Cobb (1964) has shown that certain features of echinoid behaviour could be studied by recording spontaneous

movements of the lantern and later interfering with these movements by applying various stimuli.

The recordings from the retractor muscle of the response to stimulation of the hyponeural ganglion showed a dual mechanical response. These recordings were made using a kymograph. The quick response was obtained when both the ultimate motor neurones were stimulated as well as other areas of the ganglion. The delayed response however was never obtained when the ultimate motor neurones were stimulated but only from deeper regions of the ganglion. This indicated that the delayed response was due to neural interaction within the ganglion rather than dual innervation. The experiments recording the electrical activity within the muscle cells at the same time as the mechanical responses were recorded provided further evidence for the single innervation hypothesis. These experiments showed quite clearly that the delayed response was due to the continued firing of the motor units long after the stimulus had ceased. The motor units involved in this response were the same as those involved in spontaneous movements of the muscle and in the quick response. Thus the delayed response is clearly the result of neuronal interaction within the hyponeural ganglion. Above threshold the electrical response recorded is a compound potential of many units. The electrical response proceeds the mechanical response by

60 to 70 milliseconds when the recording devices are placed at the same spot on the muscle fibre. This separation of the responses at a single point is clear evidence against the electrical responses being due to a mechanical artifact. By moving the electrode more and more distal to the hyponeural ganglion along the muscle it became apparent that the latency of response after stimulus increased proportionately to the distance moved. These experiments were refined by using two electrodes simultaneously; this confirmed the increase in latency at more distal sites of recording and allowed an approximate measurement to be made of the rate of conduction of the potential down the muscle. The rate of conduction was about 4 cms. a second and since the muscle fibres are ten to twelve microns in diameter this rules out any possibility that the contraction of the muscle is brought about by electrotonic spread of the excitation down the fibre and indicates that active conduction by spike impulses is involved. Recording with two electrodes also shows that the further from the site of stimulus the recording is made the more spread out the recorded potential is. As has been shown the potential is a compound one due to many units firing within a few tens of milliseconds of each other, however it is unlikely that all the units will conduct at exactly the same speed and therefore the farther from the

neuromuscular junction the greater the difference between the individual latencies of the fibres excited and the more spread out the compojnd potential will become.

The most difficult problem rainsed by the physiological experiments is why most of the nervous system of echinoids and asteroids is refractory to electrical stimulation. Only when the hyponeural tissue is stimulated in Echinus can a repeatable mechanical response be obtained, with one exception. This exception is when the radial cord is stimulate where it runs through the auricle of the perignathic girdle. However the response to stimulation of this area is only obtained to mechanical stimulation and never, whatever the variations in the stimulating parameters, to electrical stimulation. The response to this mechanical stimulation is in itself interesting since it clearly shows that there are separate pathways within the circumoral ring (Cobb 1964). The response to electrical and mechanical stimulus at other sites on the nervous system particularly to distal areas of the cord are completely inconsistent. At best all that can be observed are slight modifications of behaviour or the initiation or cessation of behavioural patterns. The response thus seems to depend considerably on the behavioural state which the animal is already in, this idea is contained precisely in Jordan's (1929) concept of tone although the

physiological mechanism behind it is now much better understood. This hypothesis of tone will be elaborated on in a later section.

It is noticeable when the radial cord is stimulated peripherally that tubefeet some distance away and often in radii the other side of the circumoral will indulge in some form of irregular movement though after a considerable time lapse.

Sandeman (1965) has shown in Asteroids that a compound potential can be recorded from the radial cord using wick electrodes. The significant feature of these observations is the fact that the excitation is recordable only over a relatively short distance of the cord. The wave of excitation travels at rate of 20 centimetres per second but, and this is the crucial point, it is conducted such as there is logarithmic decline in the amplitude of the response and that although excitation is carried at the most up to 50 millimetres at a dectectable level the amplitude of the response drops to half after 7 mm. Behavioural responses clearly show that waves of excitation can travel over very much longer distances and that the radial cords are necessary for the co-ordination of responses throughout the animal and hence must be able to conduct over long distances. Before an interpretation of this is

attempted one other set of observations is significant. It is equally difficult to record from echinoderm tissue as it is to stimulate it. There are only two published reports of this, that of Sandeman, as above, and that of Takahashi, 1964. The latter work was carried out on the radial cord of Diadema and demonstrates individual nerve units firing using metal-filled glass electrodes. The stimulus in the case of the work on Diadema made use of the fact that the radial cord was sensitive to light. The most difficult feature was the very long time delay observed between stimulus and the recorded action potentials, this was in the order of several seconds. During the present study many attempts were made to record with all types of electrodes from nervous tissue in Echinus and in Asterias including the use of indium-filled glass electrodes and never was any electrical activity observed. Takashashi's work must thus at present stand unconfirmed and until a more controllable parameter than light can be used as a stimulus the real significance of the results, particularly the long latency will be obscure. These results illustrate the great difficulty of recording anything at all.

Fine structure studies have made the detail of the nervous system very clear in some respects and it is this evidence that may be of help. The detail of the fine structure is discussed later but one or two points are worth anticipating. When any part of the nervous system is examined

the most striking feature is the way that the axons are tightly packed together. There are a few glial cells interspersed between the axons and in the radial cord and circumoral ring these form a lattice supporting the structure. The nervous system of the cord and the hyponeural ganglion is overlayed by a layer of epithelial cells with other cell types mixed up with it and where it is attached to the test of the animal there is a band of collagen of varying width. Most of the axons in the ectroneural tissue of the radial cord are less than 0.5 microns in diameter while those in the hyponeural tissue of both asteroids and echinoids average about 0.2 microns larger.

It was noticed when stimulating the hyponeural tissue with electrical stimulation that the response to increases in frequency of stimulation was greater up to twenty cycles per second and then dropped above this level. The same phenomenon was noticed by Pople and Ewer in the holothurian Cucumaria. The most plausible explanation of this is that at high frequency of stimulation the biochemical changes taking place affect the working of the entire system. Maturana (1960) and Horridge (1961) have both suggested that similar phenomena in other animals are due to potassium leakage flooding the whole area.

There are two main alternative explanations to account for the refractory nature of the nervous system to electrical stimulation. First, due to the nature of the nervous tissue, i.e. it's fine size, close packing, lack of separating glial tissue, etc. it may not be physically possible, with the recording and stimulating techniques used, to obtain satisfactory results. Second, as the other explanation, it may be that owing to the anatomical layout of the nervous system that only patterns of excitation are transmitted over large distances and that the various artificial stimuli applied are not of the correct type to initiate transmission over long distances.

The fact that stimulating the hyponeural ganglion with varying frequencies of electrical pulses produces the effect mentioned above indicates that at least in certain cases the physical conditions of the experiment may impose some limit upon the degree of success. It is reasonable to assume also that when the radial cord is stimulated using electrical pulses where it passes under the perignathic girdle, owing to the densely packed nerve fibres, the resistance of the tissue is high and thus a very high current voltage is required to stimulate a large area. With these high voltages it is extremely likely that much of the tissue near the nerve is cauterised and destroyed and therefore only a small area of tissue remains both uncauterised and stimulated. However, Sandeman has successfully stimulated

the radial cord in one species of urchin (although the excitation was only transmitted over relatively short distances) and also it is perfectly possible to stimulate the nerves of the hyponeural tissue in Echinus. The axons of the hyponeural tissue though of slightly larger size are not so different from the ectoneural axons as to make purely physical reason above explain the difference in the effectiveness of stimulation. On the other hand when the radial cord of Echinus is mechanically stimulated under the perignathic girdle marked responses do take place, it may therefore be that mechanical stimulation at this site excites a large number of axons simultaneously and also over a relatively long period without doing undue damage. Thus until more evidence accrues it is not possible to decide whether physical disadvantages within the experimental set-up, or the organisation within the nervous system causes the differences to both electrical and mechanical stimulation.

The same two arguments could also be applied to explain the lack of success in recording from nerves. It is unlikely however that the behaviour of the axons when examined by the more refined techniques used to record their electrical activity in echinoderms is very different from axons in other groups of animals. It therefore seems most likely that it is due to the morphological layout of the nervous system that limitations are imposed on the ability

to record successfully activity from single nervous units. The failure to record electrical activity though frustrating from the point of view of the experimenter is not so significant in itself as the failure to stimulate the nerves using electrical pulses.

Muscle Systems

Recent work has revealed that a careful re-examination of the neuromuscular systems of echinoderms will also yield many interesting features previously unsuspected. A combination of the techniques of methylene blue staining, histological sectioning and electron microscopy will be required to elucidate the organisation of a morphological system that is composed of the small diameter elements typical of this phylum. Indications of the disposition of nerve tracts can be obtained from conventional histology, but as soon as the nerve bundles begin to branch they become indistinguishable among a whole host of other types of tissue. The resolution of the light microscope is insufficient to enable the finest details to be determined. On the other hand the electron microscope will only allow examination of small areas, and cannot at present give details of the ramifications of long lengths of nerve fibres. Physiological experiments have also indicated morphological features worth examination. The fine structure studies of the muscle disclose a number of other problems requiring solution. Bargmann and Behrens (1963) were unable to find nerve fibres amongst the muscles of the ampullary wall of Asterias rubens. As one result of this finding they suggested that active conduction along and among the muscle cells may occur.

Support for this view has been provided by Prosser and others (1965) who showed that active conduction occurs in the gut muscles of the echinoid Arbacia and the present physiological studies. In the three systems described in this thesis active conduction along the length of the muscle fibre may be anticipated. Innervation in all cases is limited to a small area at one end of the fibre and propagation of excitation to other areas of the fibre must originate from here. The only specialised structure so far determined that may provide information on this subject is the T-system, thought to be concerned in active conduction in other striated muscles, and present in the striated muscles of the pedicellaria of Echinus. The T-system is found as a cylindrical network along the whole length of the muscle.

Interaction between contiguous muscle fibres is doubtful though suggested by Bargmann and Behrens (1963) on the basis of the striking peg and socket junctions between the fibres. Desmosomal connections also are extremely plentiful between cells of several types, but seem only concerned with physical bonding between the fibres, and not to subserve a physiological junction purpose (Cobb 1967 in the ampullae of Astropecten). Nexus formations or tight junctions (Dewey and Barr 1964) have not been seen in

any preparation, but further work requires to be done before conclusively establishing that these structures are totally absent. Prosser, Nystrom and Nagai's results (1965) seem to indicate passage of impulses from one muscle to another without the intervention of nerves. This may occur via a nexus.

In most typical situations the muscle fibres of echinoderms are smooth in type with a single report of striped muscle in the pedicellaria. There is however an apparent variety in size of the filaments of the smooth muscle. Muscles of special types have been noted, however, in different situations. These muscle cells contain not only myofilaments, in at least part of their length, but also large amounts of clear cytoplasm. The myofilaments show no particular orientation with regard to surrounding structures and are not attached to the plasma membrane of the muscle. The muscle cells themselves often seem unattached to any other tissue. Rosenbluth (1965) has suggested that the filaments of smooth muscle exert their force at the plasma membrane at the sides of the fibre, therefore it seems unlikely that they have a contractile function, yet the presence of typical myofilaments identifies them as muscle cells. Such muscle fibres have been described lying between the axonic layer and enveloping connective tissue in the hyponeural ganglion of Echinus (Cobb and Laverack, 1966b). In this situ tion they may act as a

cushion against stretch of the hyponeural axons during lantern movements. The neck region of the ampulla of the tube foot of Astropecten also contains cells of this kind. In this case they may yet be found to give rise to processes, and may act as a valve around the neck during walking movements.

The major corpus of the muscle fibre of the ampulla wall of Astropecten contains a considerable complement of the type of myofilaments usually associated with smooth muscle cells, but these muscle cells also extend long processes into the ampulla seam and thence to the neck of the organ. In these processes the myofilaments are spaced further apart. Although these muscle processes do insert upon a block of connective tissue the arrangement of filaments and the lack of membrane involvement in the cells seems to rule out a contractile function for these parts of the muscle fibre. The position in the ampulla seam also militates against such a function. If the processes are considered as being involved in conduction, and hence in the excitation pathway of the muscle as a result of nervous activity then a role may be proposed in which the presence of filaments at all is anomalous.

Any attempt to answer some of the questions raised by these structural observations on the muscle cells demands physiological techniques such as microelectrode recording with glass pipettes, but the fibre size of 10 - 12u

diameter means that this may be difficult particularly in view of the loose packing of these fibres. The value of the study would be great, however, in indicating whether all-or-none or graded contraction occurs in limited areas or throughout the muscle fibre.

The question of the innervation of the musculature of echinoderms is one that now has some tentative answers. Bargmann and Behrens (1963) pointed out that amongst the muscle fibres and the ampulla of Asterias no nerve fibres were seen, and yet it is apparent from the work of Kerkut (1954) and Sandeman (1965) that shocks applied to the radial nervous system invoke muscular activity in the ampulla.

Early workers on the echinoderms discussed the question of tone and though their ideas would not fit in with present physiological concepts the general idea behind their hypotheses is sound. Kerkut (1956) also commented upon this phenomenon and showed that the nervous system was capable of holding excitation at high levels over long periods even when the initial stimulus was reduced. Cobb and Laverack (1966a), Prosser (1954) and Pople and Ewer (1954) have also noted this happening in sea urchins and holothuria. It is clear that destruction of the nerves leads to a drop in the level of activity in undamaged parts of the nervous system and to a lessening in the tone of muscles.

It is obvious that there are some unusual physiological neuromuscular events to be explained in this phylum but the present studies make it increasingly clear that these events occur within the nervous system prior to the ultimate motorneurons and are not associated with either multiple innervation of single muscle cells or peculiarities of the neuromuscular junction. However the muscles themselves are very sensitive to stretch and there is evidence that some are sensitive to light. There is yet other evidence to indicate that the stretching of muscles inhibits activity in contiguous muscles, a phenomenon presumably mediated via the nervous system.

The muscle fibres of the lantern of Echinus are apparently all innervated by a single nerve fibre that ends at a neuromuscular junction enveloped in projections from the muscle fibre. The nerve at this point contains small vesicles, and the muscle fibre many large mitochondria. The presence of mitochondria in large numbers may be taken as indicative of the likelihood of a nearby junction. The single innervation occurs close to the proximal end of the muscle fibre; this is indicated by a complete absence of nerve fibres at more distal parts of the muscle, and the concentration of observed neuromuscular junctions low down along the length of the fibres.

One other feature of the innervation of the lantern

should be mentioned. When sectioned the motor tracts that run from the hyponeural ganglion to the lantern muscles are shown to contain groups of fibres of two sizes. These average 0.8 μ and 0.25 μ in diameter. The motor fibres as they ramify among the muscles have a diameter of about 0.9μ. (Within the radial system nerve fibres range in size between 0.1 and 10 μ). The smaller fibres have no observed ending. They may extend to other ancillary structures among the epithelial covering of the muscle block, or to insertion on the calcareous pieces of the lantern. We speculate that they are sensory fibres, providing some feedback from the effector organ to the nervous system.

The innervation of the other muscles mentioned, those of the tube foot and the pedicellaria show structural similarities to those of the lantern. The muscle fibres are long and thin (2 mm x 2μ) in the pedicellaria, and long and broad (5 mm x 10μ) in the tube foot ampulla. In the pedicellaria no nerves are to be found among the muscle fibres that extend between the calcareous jaw pieces. These muscles insert through pores in the jaw pieces and are innervated only within this ossicle skeleton by nerve tracts. The muscle endings and the nerve elements form an intermingled mass, and it is impossible to determine whether single, double or multiple innervation takes place. The smooth muscle and the striated muscle are innervated in the same way in this area.

The tube foot ampullary system has been the subject of some previous investigation. Bargmann and Behrens (1963) looked for electron microscope confirmation of the ribbon axon characteristic of the situation as described by Smith (1950), but were unable to find evidence for such structures. In Astropecten irregularis, the same species as that used by Smith, it is not possible to find axons of the dimensions of ribbon axons. The findings are described in an earlier section. The muscle fibres of this area are broad and are inserted on either side of the ampulla. From a small area of this main muscle mass there extends a thinner process which joins the ampulla seam that runs to the neck of the ampulla. The innervation of these muscle fibres takes place in the region of the bulb tissue where the radial nerve branch ramifies among the muscle fibre endings. These muscle tails stain with methylene blue (rongalit method) and obviously would seem to broaden out in the ampulla wall region. It is only with the electron microscope that it can be seen there is fibrous material within the cell, and that synaptic contacts occur in the ampullary bulb region. It is proposed therefore that the muscle cells and their processes are equivalent to the ribbon axons and their centrally connecting interneurons described by Smith.

This phenomena of muscle tails approaching axons as a method of innervation is not unique to the Echinodermata.

Rosenbluth (1965) has described the fine structure of a very similar system in the phylum Nematoda. Recent studies by Flood (1966) have shown the same system in Amphioxus. It is not clear whether this system represents a primitive state of the nervous system or whether the similarities are due to convergence.

It may be that the processes seen in the Astropecten ampulla preparation and in a pedicellariae of Echinus running from the muscle to the nerve are in fact extensions of the processes that enwrap the neuromuscular junction in the retractor muscle.

Although physiological evidence is missing in many cases the anatomical results lead us to suggest that as a working hypothesis the muscles of echinoderms are singly innervated at the proximal end and only at this point. In a short fibre muscle such as the longitudinal body retractor muscle of Thyone there is probably a sequential innervation throughout its length applied to the individual short fibres.

The structure of the synapses and neuromuscular junctions do not show precisely the characteristics of published pictures on these structures in other material, but are reminiscent of synapses in ctenophores (Horridge and Mackay, 1962). The synapses and neuromuscular junctions of echinoderms possess vesicles presynaptically that are

aligned against the synaptic membrane. Neuromuscular junctions reach their most complex pattern in the lantern muscle where they are enfolded; otherwise the synaptic endings seem straightforward abutments of nerve against muscle cell.

Ganglia

Bullock & Horridge (1965, Glossary) have recently defined ganglia as 'A discrete collection of nerve cells'. The term is usually applied to a nodular mass defined by connective tissue, in the periphery or in a chain separated by connectives.

By the same token neuropile is 'A tangle of fine fibres (of dendrites and axon arborizations) and their endings, quite or nearly devoid of nerve cell bodies; ... and forming much of the bulk of invertebrate ganglia. One of the most important results to be demonstrated by the fine structure studies is discrete areas of neuropile within the ganglion. Whether all three of the areas of neuropile described may be termed ganglia on the definition of Bullock and Horridge (1965) is doubtful, but they are certainly areas of nervous tissue, made up of neuropile, responsible for the co-ordination of particular organs. Within these areas of neuropile, and only there, are large numbers of structures interpreted as synapses.

The interpretation of synaptic structures is a difficult one in this material (see also Graziadei 1965). A considerable number of specimens was observed to try and elucidate the structure. Such material was examined after each of the fixation techniques mentioned in the methods and materials section. There is little difference observable

between them save for a diminution of electron scattering by the unit membranes of vesicle at the synapse after PTA treatment.

As a generalization the so-called synaptic contacts can be considered as falling into two classes. Those with patent cleft between pre- and post-synaptic axons, and those without. Gray & Young (1964) state that considerable attention should be paid to the membrane specializations rather than concentrations of vesicles pre-synaptically. In hyponeural ganglia the synaptic regions are densely packed with axons and in many places the abutting membranes appear as a single thick structure which has not yet been demonstrated as composed of two distinct cell membranes.

Most of the synaptic contacts seen have no cleft and showed a diffuseness of the membranes of the area. This was a regular feature and was eventually assumed to be the normal situation. At the present time it is not possible to distinguish between the possibility of complete fusion of the pre- and post-synaptic membranes in this region (in a different manner to the nexus of Dewey & Barr (1962) or whether there is some structural material lying between the two membranes in the supposed cleft region. From consideration of many examples it seems that those positions exhibiting a cleft between the axons may be just short of the real area of synaptic contact.

A concentration of small vesicles may be another criterion for the occurrence of a synapse. These are not so readily visible after PTA treatment. They are, none the less, numerous at certain sites, orientated relative to the membrane.

At the moment there is no information as to the mode of action of these synapses. No evidence has accrued to suggest the involvement of 'synaptic vesicles' in transmission, little is known of transmitter substances in echinoderms, and the structural specialization of the contact membranes may indicate an electrical transmission. If electrical transmission does take place the part played by vesicles is unexplained; membranes showing diffuseness are seen at sites other than vesicular ones. This state of affairs is comparable to those described by Horridge and Mackay (1962) in a cnidarian.

The sites of interaction occur most commonly between adjacent parallel motor fibres, between small fibres and also between sundry other unidentified fibres. Synapses onto cell bodies or axon hillocks have not been seen. They appear localized in the neuropile.

It is however difficult to interpret the vesicle filled axons found in the ectoneural tissue of the radial cord of asteroids found against the collagen tissue separating the ectoneural and hyponeural tissue. It is

unlikely that the transmitter could pass from these axons through the collagen layer to the hyponeural tissue (although the same situation is present between the ectoneural and hyponeural tissue of the hyponeural ganglion in the lantern of Echinus). There seems to be no synaptic connection between the vesicle filled axons nor with other axons of the ectoneural tissue. Their function and mode of operation must remain unexplained but perhaps two features should be borne in mind. First they occur at the boundary of the hyponeural tissue with the ectoneural tissue and second there are almost certainly no nerve tracts within the cord joining the two levels of nerve tissue, as described by other authors, (see Nichols, 1966).

The study of the radial cord of Astropecten has shown that the cord consists of a very large number of axons mostly of a fairly uniform diameter running lengthways up the cord. There only appear to be numerous cell bodies within the hyponeural tissue and thus most of the axons within the ectoneural have their cell bodies distal to the radial cord itself. Within the cord of asteroids (but not in echinoids, Cobb unpublished) discrete areas of neuropile occur adjacent to the tubefeet. These areas of neuropile are presumably responsible for the co-ordination of the behaviour of the tubefeet. Due to the fact that in asteroids there are no branches of nerves to the tubefeet

it is proposed that the areas of neuropile are part of the tubefoot system and not part of the radial cord itself. In echinoids where branches to the tubefeet are present there are no areas of neuropile within the cord although there is as yet no evidence to show that they in fact occur at the tubefeet either.

In the pedicellariae it has been shown that there is an area of neuropile within each jaw of these structures. The nerves forming the neuropile arise from sensory cells at the faces of the jaws but it is not clear whether there are interneurones within the neuropile. The innervation of the smooth and striped muscles that close the jaws is mediated via axons from this neuropile.

Thus the fine structure studies have shown each organ to have a controlling ganglion and that the rest of the nervous system, i.e. the cords and the circumoral ring are just trunks of axons, sensory, motor, and inter-neurones, quite devoid of synaptic contacts.

Sensory Cells

Comparison of versene treated decalcified material with untreated material revealed no major differences in the fine structure that were significant in the study of the sensory cells.

There are two types of epithelial tissue in Echinus. The first forms a microvillous surface and occurs widely over the external surface of various organs; it is also present externally on echinoderm larvae (Laverack, 1964 unpublished). The second type of epithelial cell is described in Cobb and Laverack (1966b) and is found covering certain areas of nervous tissue, and lantern muscles and lining the lumen of the ampullae. These epithelial cells have a large nucleus in relation to the total cell size and possess microvilli only around the single cilium and not all over the surface. The pedicellariae are covered with the microvillous epithelial cells described in this paper. In two areas examined the epithelial cells are compressed so that the external surface of them consists only of a single ring of microvilli surrounding a cilium.

The sensory hillock of the globiferous pedicellariae consists of the laterally compressed epithelial cells. The hillock can be shown to be sensitive to mechanical stimulation and as only the ciliated cells described above

are present in this area these are presumably the receptors involved. On the inner faces of the jaws of the tridentate pedicellariae there are, laterally, narrow tracts of cilia. These cilia arise from a similar compressed epithelial cell to those on the sensory hillock. The sensory regions of the jaws are not however confined to these tracts of cells. There are no other cell types present on the rest of the jaws and for this reason the general epithelial cells must serve a sensory function. The fact that all these epithelial cells give rise to axonic processes supports this view and would seem to indicate that the distribution of sensory cells is syapatric with the wide distribution of the epithelial cells. It is interesting to note in Astropecten that even the cell bodies of the central distributory neurons of the hyponeural tissue of the radial cord are sometimes ciliated (Cobb, unpublished). The epithelial cells are seemingly multifunctional and contain a wide variety of inclusions generally associated with non-sensory functions. At the present stage in the study of the pedicellariae nothing is known of the connexions centrally within the jaw but it is possible that due to the morphological distribution in the nerve mass of the sensory axons some receptor cells are more important functionally than others.

The cilia of the sensory hillock shows no obvious modifications from typical motile cilia in other organisms

and other places in echinoderms which could account for their being associated with a sensory cell. The possible role of these cilia and their thickened microvilli in the transduction of the tactile stimulus to an electrical response is not clear from a fine structure study. Recent behavioural studies by Campbell (unpublished) on gemmiform pedicellariae have shown responses to tactile stimulation of the sensory hillock are little different from responses to tactile stimulation of other areas of the jaws. It therefore seems likely that the sensory hillock may also have sensory functions of a non-tactile nature.

The apparent connexion of the neurotubules in the axon to the basal body of the cilia may have interesting functional significance.

Echinoids are known to be sensitive to chemicals, light and touch and also to show geotropic responses and it seems possible that the epithelial cells of the pedicellariae may have varied sensory functions. The cells of ocelli described by Eakin and Westfall (1964) and those found in the statocyst of the holothurian Synaptula (Laverack, unpublished) are clearly derived from the general epithelial cells and the present study describes tactile sensory cells derived from the same source. It seems possible that these regions may be formed by cells specialising in functions already occurring in the general epithelial cells. Other

cells specialising in different functions seem to be responsive to certain stimuli; muscles in some echinoids are considered to be sensitive to light (Boltt and Ewer, 1963) and sensitive to stretch (Cobb and Laverack, 1966a) as are axons (Millott & Takahashi, 1963).

The significance of the potency of various stimuli on different types of cells in echinoderms in the formation of behavioural responses is obscure. It is clear however from the observation that axonic processes arising from seemingly all microvillous cells, at least, in the pedicellariae, that an extensive nervous system is involved in the co-ordination of responses.

CONCLUSIONS

Von Uexkull called the echinoderms a republic of reflexes. Hyman "salutes the echinoderms as a noble group especially designed to puzzle the zoologists". These two statements sum up the whole of the problems confronting the investigator of echinoderms. Not only do the echinoderms function by making use of many peripheral organs all relatively independant of one another, but many of the techniques used successfully in the study of the anatomy and physiology of other animals fail to work on echinoderms.

The most important point brought out by the present work and one not emphasised enough in the general literature on echinoderms, is that they differ from nearly all other triploblastic animals in that they do not show cephalisation. This lack of cephalisation is forced upon them due to the pentamerous symmetry of the animals. In embryo they have a normal bilateral symmetry and there is some evidence to show that there is still slight dominance within the pentamery of this original bilateralism, Knight-Jones, (1964). Nevertheless there is no single or paired central ganglion that can be termed a brain within the phylum. This idea of no cephalisation within the phylum underlies the proposed hypothesis in this thesis, albeit a very tentative one, to account for the co-ordination of behaviour in echinoderms.

Because the echinoderms do not possess a cerebral ganglion, due to their discarding cephalisation for pentamery and hence 'a central nervous system' however primitive, does not of itself preclude that areas of nerves responsible for co-ordinating exist. Early workers though occasionally using the word ganglion in a loose terminalogical sense have not described any such defined areas. This study however has shown the presence of three such areas, one physiologically and anatomically and the other two only anatomically. However, in the last two cases the presence of a ganglia can be inferred from behavioural observations. These ganglia, containing areas of neuropile, and thus many synapses, are described from three entirely different organs of echinoderms and allow the inference to be made that all organs and organelles and sometimes discrete parts of organs have their own controlling ganglion. Therefore from the relatively complex structure of a pyramid of the lantern with its many sets of muscles to the smallest stem of a pedicellaria (in this case part of an organelle) the presence of a controlling nerve centre can be anticipated. The morphology of much of the asteroid system has been described by Smith, (reviewed 1955,) using methylene blue staining techniques. The electron microscope, which was not available to him at the time, is revealing some of the study

to be in need of re-interpretation. The basic descriptions of Smith however are of great value in examining nerve muscle systems with the electron microscope and should direct future workers onto many interesting preparations. Electron microscopy has been carried out by a few experimenters, notably Bargmann and Behrens, (1963), Eakin and Westfall, (1964), and Kawaguti, (1962-66). Unfortunately although the work of the last author is most extensive, it is of poor standard, possibly due to bad presentation but nevertheless of dubious use while unconfirmed, and often lacking in detail.

Echinoderms clearly have an extensive nervous system and this is shown by gross histological and electron microscope studies. The units comprising the nervous system are in many respects similar to comparable units in other phyla. The axons have the same appearance, contain the same inclusions of vesicles, tubules, small mitochondria etc. as axons from other animals. There are no obvious specialisations rather the reverse, with the axons jammed closely together in a naked mass with few glial cells or other interstitial cells; the same simplicity is seen in the synapses and the neuromuscular junctions. Although the small size of the axons and other factors discussed earlier make physiological experiments difficult there is no reason to suspect at present that the individual nerve units work any differently

in echinoderms than do axons in other animals, at least as far as the broad principles are concerned.

There are four vital points to come out of the fine structure studies. The first is that the nervous system is very extensive and this fact alone militates that it is of great importance in the co-ordination of the behaviour of echinoderms. The second point is that there are extensive areas of neuropile, and most significantly these areas can be precisely located; outside these areas there are few if any synapses. Third many epithelial cells, probably the majority and possibly all give rise to axons which feed into the general nerve plexuses and into the radial cord etc. This may only apply to the microvilleus epithelial cells but it is interesting to note that some of the cell bodies of the axons of the hyponeural tissue in the radial cord of Astropecten are ciliated. Finally there are clearly neuro-muscular junctions present and although these are somewhat unusual they do not confirm the original suggestion of ribbon-like axons enveloping the muscle. In the same context there are so far no indications from fine structure work that any contacts exist, between cells of any type, that may facilitate electrical transmission of excitation from one cell to another.

The number of authors publishing on the physiology of echinoderm nerve and muscle systems is strictly limited. The work of Ewer and his co-workers is most important (1954,

55, 58, 63 a and b), the work of Millott and others working with him is interesting, though mostly confined to a small part of a system. Smith, Bullock, Sandeman, Takahashi and Prosser have all contributed.

From the physiological studies there are again some important conclusions to be drawn. First the dual response noted in some nerve muscle preparations is due to nervous interaction and not dual innervation. Second, muscle cells can almost conclusively be shown to conduct action potentials. Third, it can be shown that there are set pathways within the nervous system and not just a simple nerve net system. Lastly, the failure of certain experiments in recording electrical activity indicate certain structural features within the layout of the nervous system which account for such failures.

The sensory input of the nervous system can again be deduced from a study of isolated systems. Clearly many of the epithelial cells throughout the animal have a sensory function (they may well also have other functions) and these cells contribute axons to the nerve plexus of the animals and also to the radial cord and circumoral ring. Bullock (1965) recently considered the spread of excitation in echinoids and asteroids judged by the response of various organs on the test and considered anatomical layouts that could account for this. He demonstrated that clearly a simple

nerve net, such as the coelenterates have, is not present and in echinoids, at least, came to the conclusion that from each point of stimulus a number of axons each ran in a straight line away from that point rather like the spokes of a cartwheel. This is apparently a rather uneconomic system but he showed that mathematically it was at least possible.

It is regretable that the present study does little to clarify this situation because it is not possible as yet to trace single axons over long distances using the electron microscope nor is it as yet possible to study the fate of a single axon in an area of neuropile.

Kinosita (1941) claimed, though Bullock (1965) failed to confirm this that excitation could in fact be spread around corners and the work of Millott among others has shown that the behaviour of echinoderms (in this case echinoids) changes with the presence or absence of the radial cord. Although there are discrepancies in detail these findings show two things, first that there is direct connection between sensory cells and the controlling ganglion of an organ and second that there is connection between the various different ganglia. In the lantern retractor muscle system of Echinus it has been shown both anatomically and physiologically that the muscle cells must conduct excitation along their length actively by spike potentials from the neuromuscular junction, and in two other systems

this is implied from the anatomical layout alone. The motor fibres in one system run from the ganglion to the muscle and are there enfolded in short processes from the muscle at the neuromuscular junction. In the other two systems, to a differing degree, the muscle fibres extend processes of some length which pass towards the area of neuropile, and in one case right into it, to form neuromuscular junctions with axons. It has not been possible so far to study the connections of the motor axons within the ganglia but two features can be implied from physiological and anatomical knowledge. First some means of spreading the excitation carried originally into the ganglion by only a few sensory fibres so that all motor axons can, if necessary, be fired almost simultaneously. Second, not only is there a direct response to stimulation from the motor neurones there is also a lasting excitation within the ganglion that produces bursts of electrical activity within the motor axons for long periods after the stimulus has been withdrawn. Therefore some area within the ganglia must be responsible for the creation of this lasting excitation.

Work by Smith (1950) and Kerkut (1954) on the locomotion of asteroids and work by Cobb on the movements of the lantern of Echinus (1964) have shown the involvement of a pacemaker in the nervous system. Millett has also

described such a pacemaker in the radial cord of Diadema. It is possible that all ganglia have a pacemaker present within them and that these are subject to dominance from adjacent pacemakers in other organs and in certain cases organs from all over the animal are subject to dominance from a single pacemaker. The work on the lantern of Echinus has shown that the effect of a pacemaker is more marked in some ganglion-motor neurone-muscle systems at any one time than in others, even though all the systems are in the same phase of rise and fall of excitation. Changes in the dominance of the pacemakers can be clearly seen in the lantern preparation with irregular rhythm occurring during the change followed by a variation in the amplitude within the individual ganglion-motor neurone-muscle systems. The same phenomenon is shown in the changes of dominance within the arms of a starfish during the walking movements.

It is now possible to present an overall hypothesis to account for the co-ordination of behaviour in echinoderms. The test and many of the organelles of echinoderms are covered by an epithelium where the great majority of the cells have a sensory function and give rise to sensory axonic processes. These sensory axons pass into ganglia of various sizes, depending on the size of organelle they serve. The ganglia are also under the influence of a pacemaker system, which involves dominance between all the ganglia to a varying degree. The excitation from the pacemaker and the imput

from the sensory system maintain the ganglia and hence the organ at a certain level of excitation, equivalent to Jordans (1929) tone. The level of excitation within the ganglion can also be influenced by imput via interneurones from other ganglia (as explained below) and these three sources of excitation shape both the long term and short term responses of the organelle. Under certain circumstances the sensory information passing directly from the epithelium to the ganglia is passed on from the ganglia via set pathways to other ganglia and again under certain conditions passed on yet again to other ganglia and hence passed throughout the nervous system. However in many cases the sensory information never passes the first ganglia and the field of excitation from a point of stimulus under these conditions would be in straight lines from the stimulus along the sensory axon to the ganglia, accounting for Bullock's (1965) observations. It is equally clear however under certain conditions, probably dependent on the tone of the ganglia as described above that sensory information can be transmitted for long distances via numerous ganglia. Sandeman's (1965) results are explained by the fact that the stimulus used was applied to many axons all running between ganglia or direct from sensory cells, these axons conduct the impulse to the ganglion to which they run, which is not the full length of the cord. Thus in any cross section of

the cord stimulated the axons present run for varying lengths down the cord, the majority for only short distances and hence as more and more axons run to ganglia the farther from the point of stimulus one travels the smaller becomes the compound potential. This explains the logarithmic fall off of the potential. After the stimulated axons reach the ganglion, they may stimulate the organ that is associated with the ganglion or send excitatory impulses via interneurones to other ganglia down the cord. However due to neural delay within the ganglia, due to synapses, and the anatomical layout of the nervous pathways to the ganglia, the impulses that leave the ganglia via interneurones to other ganglia are not co-ordinated throughout the cord and hence no compound potential exists. To record single units of activity is very difficult and therefore the post-ganglionic firing of interneurones would not be detected. This explains Sandemans observations of physical responses with no concurrent electrical activity. In the radial cord of Echinus the refractoriness of the cord is explained by the fact that the electrical stimulus applied to the cord is not of the correct pattern to stimulate further ganglia. In certain cases mechanical stimulation succeeds in doing this where electrical stimulation fails, clearly indicating that it is physical limitations of the electrical stimulus that fails

to excite the nervous system and not the nervous system itself.

The fact that muscle cells sometimes develop processes that penetrate the nervous system is not of major importance in understanding how echinoderms are co-ordinated, but it is interesting that both such muscle cells and epithelial cells fulfill the roles of nerves in similar situations in other groups of animals. It would thus seem that the main function of the nerves in echinoderms is to co-ordinate responses and the epithelial cells and the muscles cells contribute much more to reflex arcs than they do in other phyla.

In a detailed examination much still remains obscure but in general principles the co-ordination of echinoderms can be regarded in a new light. Individual organelles respond to stimulus via a co-ordinating ganglion and the response depends on three things; first the direct sensory imput to the ganglion, second the level of excitation within the ganglion from a pacemaker system and third the level of excitation reaching the ganglion via interneurones from other ganglia. The balance of the levels of excitation of the three give rise to the response and also to the general tone of the organelle when not actively stimulated. This explains why in many cases the response to a single

precise stimulus varies so widely. The whole animal is co-ordinated using the pacemaker system, with the implication of dominance, and the interneurones between ganglia. Thus in the locomotion of asteroids the interneurones and the pacemaker ensures stepping locomotion in a set direction and the local sensory imput to the ganglia of each effector organ allows flexibility within the system to account for the constantly changing environment of a moving animal. If the environment changes adversely the sensory imput stimulates the interneurones between ganglia to fire and hence changes the overall pattern of excitation to change including if necessary the dominance. As stressed earlier the details of this system at a unit level are not clear, for example the interneurones between ganglia may well be directly involved with the pacemaker system and there is some evidence that an inhibitory system may be involved and nothing is known of proprioceptive imput from the organs (though in the last case no actual structures apparently exist). It may well be that since muscle has been shown to be sensitive to stretch and possibly to light, Boltt and Ewer (1963) that in some cases reflex responses occur without involving the nervous system at all, nevertheless the theory propounded above does allow for experimental testing and fits many of the known facts.

The system is clearly highly advanced from the nerve nets of coelenterates. It is not yet clear however whether this system represents the basic nervous system from which the anterior oriented nervous system of animals showing cephalisation have developed or whether it is a secondary development due to pentamerism. If the former a fuller understanding could lead to far-reaching conclusions about integration in nervous systems in general and if the latter allow some interesting observations to be made on a fairly sophisticated nervous system involving relatively few units.

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